For Reference

NOT TO BE TAKEN FROM THIS ROOM

Ex libris
Universitatis
Albertaensis

QUAECUMQUE VERA

Digitized by the Internet Archive
in 2021 with funding from
University of Alberta Libraries

https://archive.org/details/Trip1975

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR Everard Martin Trip

TITLE OF THESIS I. Synthesis of Cytokinin Analogues of
N^{6}-(3-Methyl-2-butenyl)adenosine.
II. Stereoselective Synthesis of Alkyl 2-
Deoxy-β-*D*-*erythro*-pentofuranosides and
2'-Deoxyadenosine.

DEGREE FOR WHICH THESIS WAS PRESENTED Ph.D.

YEAR THIS DEGREE GRANTED Fall, 1975

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

THE UNIVERSITY OF ALBERTA

I. SYNTHESIS OF CYTOKININ ANALOGUES OF *N*^{6}-(3-METHYL-2-BUTENYL)ADENOSINE.

II. STEREOSELECTIVE SYNTHESIS OF ALKYL 2-DEOXY-β-*D*-*ERYTHRO*-PENTOFURANOSIDES AND 2'-DEOXYADENOSINE.

by

EVERARD MARTIN TRIP ©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA

FALL, 1975

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled

I. Synthesis of Cytokinin Analogues of $\underline{N}^6$-(3-Methyl-2-butenyl)adenosine.

II. Stereoselective Synthesis of Alkyl 2-Deoxy-β-D-erythro-pentofuranosides and 2'-Deoxyadenosine.

submitted by Everard Martin Trip in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

TO MY MAM AND DAD

WHO WANTED ME TO

GET AN EDUCATION

ABSTRACT

A number of nucleoside analogues of the cytokinin *N*^{6}-(3-methyl-2-butenyl)adenosine and their corresponding biologically analogous *N*^{6}-benzyl derivatives have been prepared. Alkylation of adenosine, 2'-deoxyadenosine and 9-β-D-arabinofuranosyladenine by 3-methyl-2-butenyl bromide or benzyl bromide gave the *N*^{1}-alkylated derivatives which underwent base catalyzed rearrangement to the *N*^{6}-substituted compounds. *N*^{6}-Benzyl-3'-deoxyadenosine was also prepared from 3'-deoxyadenosine in a similar manner. The extent of alkylation by 3-methyl-2-butenyl bromide was found to be greatly increased by the addition of nonnucleophilic acid acceptors. Stannous chloride catalyzed sugar methylation of *N*^{6}-(3-methyl-2-butenyl)- and benzyladenosine gave the corresponding 2'-*O*- and 3'-*O*-methyl derivatives. *N*^{7}-substituted formycin derivatives were prepared by reaction of 7-chloro-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine with 3-methyl-2-butenylamine or benzylamine. Sulphur alkylation of 6-mercaptopurine riboside and 6-thioguanosine by 3-methyl-2-butenyl bromide gave the *S*^{6}-(3-methyl-2-butenyl) derivatives of these compounds. Certain aspects of the mass spectral fragmentation patterns and nmr spectra of these cytokinin analogues are discussed.

A route for the stereospecific synthesis of alkyl

2-deoxy-β-D-*erythro*-pentofuranosides has been developed. Benzyl and phenyl 2-*O*-methanesulphonyl-3,5-di-*O*-benzyl-1-thio-α-D-arabinofuranosides were prepared from D-arabinose *via* 1,2-*O*-isopropylidene-β-D-arabinofuranose and 3,5-di-*O*-benzyl-D-arabinofuranosyl chloride. In reactions of the benzyl-1-thio compound with methyl, ethyl or *iso*-propyl alcohol in the presence of barium carbonate, stereospecific attack of the alcohol at *C*-1 of the sugar, with concomittant migration of the benzylthio group to *C*-2 and displacement of methanesulphonate, gave the methyl, ethyl and *iso*-propyl-2-*S*-benzyl-3,5-di-*O*-benzyl-2-thio-β-D-ribofuranosides in good yield. Subsequent desulphurization with accompanying debenzylation occurred using Raney Nickel to give high yields of the corresponding 2-deoxy-β-D-*erythro*-pentofuranoside derivatives.

Application of this procedure to the synthesis of 2'-deoxynucleosides was less successful. Condensation of the 1-benzylthio-2-*O*-methanesulphonyl sugar with 6-chloropurine gave moderate conversion to the 9 and 7-isomers of 6-chloro-(2-benzylthio-3,5-di-*O*-benzyl-2-deoxy-D-ribofuranosyl)purine. β-Anomers were the predominant products, but both positional isomer fractions contained small amounts of the corresponding α-anomers. Amination, desulphurization and debenzylation of the 9-isomer gave 2'-deoxyadenosine and a very

small amount of 9-(2-deoxy-α-*D*-*erythro*-pentofuranosyl)adenine. Amination of the 7-isomer gave the β and α-anomers of 7-(2-benzylthio-3,5-di-*O*-benzyl-2-deoxy-*D*-ribofuranosyl)adenine. Desulphurization and debenzylation of the β-anomer gave 7-(2-deoxy-β-*D*-*erythro*-pentofuranosyl)adenine which is the 7-isomer of naturally occurring 2'-deoxyadenosine. The deoxynucleosides were obtained in poor overall yield due largely to the low and unpredictable efficiency of the desulphurization reaction using Raney Nickel.

ACKNOWLEDGMENTS

I would like to thank all those people who have made my stay at the University of Alberta a happy and enlightening experience. My fellow graduate students and postdoctoral fellows have made working here a pleasure rather than a chore and have provided many interesting discussions and arguments about all aspects of life, including chemistry. My research supervisor, Dr. M. J. Robins, has been especially helpful in providing constant encouragement, advice and ideas throughout the course of this work.

Special thanks are due to Miss Walia Halim for the distillation of many litres of dry solvents, to Miss Diane Dowhaniuk for deciphering and typing this thesis and to the staffs of the various service laboratories, workshops and storerooms of this department for their extensive assistance and advice.

I am grateful to the trustees of the Izaak Walton Killam Memorial Fellowship, the National Research Council of Canada and the University of Alberta for generous financial assistance during this work.

Finally, I would like to thank my wife, Barb, for the love which she has given me and without which I would not have been able to survive.

TABLE OF CONTENTS

Page

ABSTRACT v

ACKNOWLEDGMENTS viii

LIST OF SCHEMES xi

LIST OF TABLES xiii

GENERAL INTRODUCTION 1

CHAPTER I Synthesis of Analogues of N^6-(3-Methyl-2-butenyl)adenosine and Other Cytokinin-Related Nucleosides

INTRODUCTION

A. 6-N-Substituted-6-amino-purines Having Cytokinin Activity 7

B. The Occurrence of Cytokinins in Transfer Ribonucleic Acids 14

C. The Biological Acitivity of Cytokinins 19

RESULTS AND DISCUSSION 23

A. Synthesis of Analogues of IPA . . 24

B. Mass Spectral and NMR Observations 35

EXPERIMENTAL

A. General Procedures 50

B. Syntheses 52

Page

CHAPTER II Synthesis of 2'-Deoxynucleosides and 2-Deoxyribofuranosides

INTRODUCTION 75

RESULTS AND DISCUSSION 87

A. Preparation of Phenyl and Benzyl 3,5-Di-*O*-benzyl-1-thio-α-*D*-arabinofuranosides 90

B. Reactions of Benzyl and Phenyl 3,5-Di-*O*-benzyl-2-*O*-methanesulphonyl-1-thio-α-*D*-arabinofuranoside . . . 107

EXPERIMENTAL

A. General Procedures 124

B. Syntheses 127

REFERENCES 171

LIST OF SCHEMES

Scheme	Description	Page
I	Synthesis of *N*^{6}-Substituted Adenine Nucleosides *via* *N*^{1}-Alkylated Derivatives.	25
II	Self Condensation of 3-Methyl-2-Butenyl Bromide.	28
III	Synthesis of 2' and 3'-*O*-Methyl Derivatives of *N*^{6}-(3-Methyl-2-butenyl) and *N*^{6}-Benzyladenosine.	32
IV	Reaction of 7-Chloro-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine with 3-Methyl-2-butenyl and Benzylamine.	33
V	Synthesis of 6-*S*-(3-Methyl-2-butenyl) Derivatives of 6-Mercaptopurine Riboside and 6-Thioguanosine.	34
VI	Mass Spectral Fragmentation Ions of *N*^{6}-Substituted Adenine Nucleosides.	36
VII	Mass Spectral Fragmentation Pathways for 6-*S*-(3-Methyl-2-butenyl)-6-thiopurine Nucleosides.	42
VIII	Synthesis of Purine Nucleosides by the Fischer-Helferich Procedure.	76
IX	Synthesis of Pyrimidine Nucleosides by the Hilbert-Johnson Procedure.	78
X	Synthesis of 2'-Deoxynucleosides by Base-Sugar Coupling Procedures.	80
XI	Glycoside Syntheses Involving Ribofuranose Derived 1,2-Anhydro and 1,2-Episulphonium Ion Intermediates.	88
XII	Syntheses of 1,2-*O*-Isopropylidene-β-D-arabinofuranose (77).	92
XIII	Synthesis of 3,5-Di-*O*-benzyl-D-arabinose (79).	96
XIV	Synthesis of Ethyl 3,5-Di-*O*-benzoyl-1-thio-α-D-arabinofuranoside (83).	98

Scheme	Description	Page
XV	Reactions of 3,5-Di-O-benzyl-D-arabinofuranosyl Chloride (86).	100
XVI	Synthesis of the α and β-Anomers of Phenyl 3,5-Di-O-benzyl-1-thio-D-arabinofuranose (87 and 88).	104
XVII	Synthesis of Phenyl and Benzyl 2-O-Methanesulphonyl-3,5-di-O-benzyl-1-thio-α-D-arabinofuranosides (87 and 88).	107
XVIII	Reactions of Phenyl 2-O-Methanesulphonyl-3,5-di-O-benzyl-1-thio-β-D-arabinofuranoside (93) with Alcohols.	109
XIX	Synthesis of Alkyl 2-Deoxy-β-D-erythro-pentofuranosides (104-106).	111
XX	Synthesis of (2-Benzylthio-3,5-di-O-benzyl-2-deoxy-D-ribofuranosyl)adenine Nucleosides (113-115).	116
XXI	Synthesis of (2-Deoxy-D-erythro-pentofuranosyl)adenine Nucleosides (10, 62 and 118).	120

LIST OF TABLES

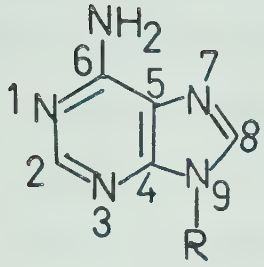
Table	Description	Page
I	Characteristic Mass Spectral Ions of 3-Methyl-2-butenyl Analogues.	37
II	Characteristic Mass Spectral Ions of Benzyl Analogues.	38
III	Chemical Shifts of Sugar Hydrogens.	45
IV	Coupling Constants of $\underline{C}_1$, Hydrogens.	46
V	Optical Rotations of some 1-Thio-D-arabinofuranoside Derivatives.	101

GENERAL INTRODUCTION

In 1871 Miescher[1)] isolated a material from the nuclei of pus cells which he called nuclein. The term "nucleic acid" was subsequently introduced by Altmann[2)] to describe this material and he and others developed general methods for the isolation of nucleic acids from a number of sources.

The nucleic acids are polymeric chains of nucleotides (composed of a purine or pyrimidine base, a furanose sugar component and a phosphoric acid residue) linked by phosphodiester bonds. They fall into two distinct classes, depending upon the nature of the sugar moiety present: ribonucleic acid (RNA) in which the sugar is D-ribose and deoxyribonucleic acid (DNA) which contains the sugar moiety 2-deoxy-D-ribose (2-deoxy-D-*erythro*-pentose). The identities of the sugar residues in the nucleic acids were determined during the years from 1910 to 1930 by Levene and coworkers.[3,4,5)]

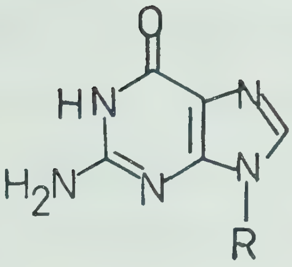
Five heterocyclic bases are commonly found in the nucleic acids and were identified in the late 19th century by Kossel, Schulze and Fischer, among others.[6)] The purines adenine (1) and guanine (2) and the pyrimidine cytosine (3) are found in both DNA and RNA. The remaining pyrimidine base normally found in DNA is thymine (4) and in RNA is uracil (5).



1 R = H

6 R = R′

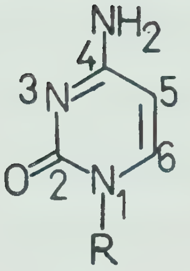
10 R = R″



2 R = H

7 R = R′

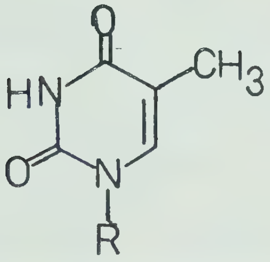
11 R = R″



3 R = H

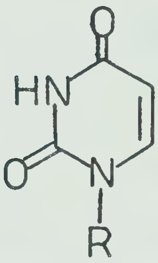
8 R = R′

12 R = R″



4 R = H

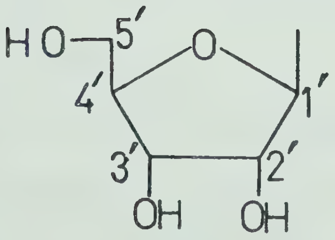
13 R = R″



5 R = H

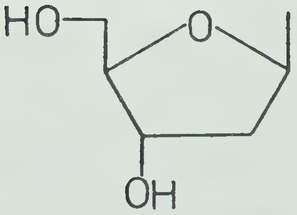
9 R = R′

R′ =



D-RIBOSE

R″ =



2-DEOXY-D-RIBOSE

In 1909 Levene and Jacobs[7)] introduced the term nucleoside to describe the carbohydrate derivatives of purines and pyrimidines isolated from alkaline hydrolysates of yeast nucleic acid. This term is now widely used to include all compounds of natural or synthetic origin which contain a heterocyclic base linked to the C-1 position of a sugar. The commonly occurring nucleosides found in RNA are adenosine (6), guanosine (7), cytidine (8) and uridine (9), and in DNA are 2'-deoxyadenosine (10), 2'-deoxyguanosine (11), 2'-deoxycytidine (12) and thymidine (13).

In addition to the commonly occurring nucleosides named above, many other modified nucleosides have been found in nucleic acids in minor amounts.[8)] Modifications are generally restricted to the base residue, and often consist of simple methylation. As well one type of sugar modified nucleoside, the 2'-O-methyl derivative is known.

The position of attachment of the sugar to the base in naturally occurring nucleosides was first deduced by chemical methods,[9,10)] but it was later shown that assignment could be made by simple ultraviolet spectroscopic comparisons.[11,12,13)] The furanose nature of the ribose ring was demonstrated by Levene and Tipson[14)] and was later verified by periodate oxidation studies.[15,16)] Todd and coworkers also used periodate oxidation to pro-

vide the first indication of configuration at the anomeric carbon.[17)] The same group later confirmed the assignment of the β-configuration when they discovered the formation of cis-intramolecularly linked 5'-cyclonucleosides derived from cytidine (8) and adenosine (6).[18)] Final structure proof, synthesis by an unambiguous route, has also been carried out by Todd and coworkers for all of the common nucleosides.[19)] The foregoing material has been the subject of several extensive reviews.[20,21,22,23,24)]

Nucleic acids then are composed of individual nucleosides joined together through their 3'- and 5'-positions by phosphodiester linkages.[25)] DNA is the primary genetic material and it is the order of the bases in the DNA molecule that furnishes the genetic information.

The living cell contains three main kinds of RNA. Messenger RNA (mRNA) is transcribed directly from the DNA and has a base sequence complementary to that in the DNA.[26,27)] The mRNA thus acts a carrier of the genetic information contained in the DNA. Transfer RNA (tRNA) serves the purpose of translating the information carried by the mRNA into the synthesis of specific proteins in the cell. An amino acid can be attached to the 3'- end of a tRNA molecule by an ester linkage. There are numerous different tRNA molecules, each one being specific for a particular amino acid.[28)] One region of the tRNA,

known as the anticodon, consists of a sequence of three bases which is characteristic for the amino acid carried by the tRNA.29) Because of the specific base pairing that occurs between guanosine (7) and cytidine (8) and also between adenosine (6) and uridine (9) or thymidine (13),30,31) the triplet of bases in the anticodon of the tRNA pairs, in general, only with its complementary sequence of three bases on the mRNA.32,33) The mRNA is thus divided into a sequence of base triplets, termed codons, each of which will pair only with a specific tRNA. In this way the sequence of bases in the mRNA defines the sequence of amino acids in the protein to be synthesized. This coding of base triplets for specific amino acids is known as the genetic code.34,35,36)

Ribosomal RNA (rRNA) is the most abundant form of RNA in the cell and is found in particles called ribosomes. The ribosomes move along the mRNA molecule and "read" the sequence of codons. As they do so, tRNA molecules having the required anticodon migrate to the mRNA-ribosome complexes and donate their amino acids to the growing polypeptide chain. Thus as a ribosome travels from the beginning to the end of a mRNA molecule, a complete polypeptide, having the desired amino acid sequence, is synthesized.37,38,39) The above description of the biological function of the nucleic acids is extremely brief and a convenient overview of this subject

has been compiled by Davidson.40)

Of the types of nucleic acid described, tRNA is the richest source of modified nucleosides. Of particular interest to this work is the tRNA-derived modified nucleoside N^6-(3-methyl-2-butenyl)adenosine, which forms the basis for Chapter I of this thesis. Chapter II is concerned with the development of a route for the stereospecific synthesis of 2-deoxy-furanosides and 2'-deoxy nucleosides.

CHAPTER I

SYNTHESIS OF ANALOGUES OF $\underline{N}^6$-(3-METHYL-2-BUTENYL)ADENOSINE AND OTHER CYTOKININ-RELATED NUCLEOSIDES.

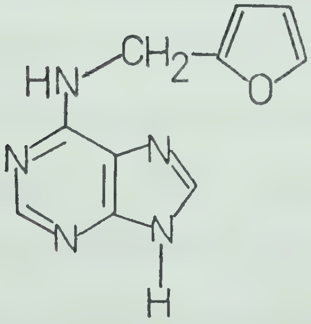
INTRODUCTION

A. 6-N-SUBSTITUTED-6-AMINO-PURINES HAVING CYTOKININ ACTIVITY.

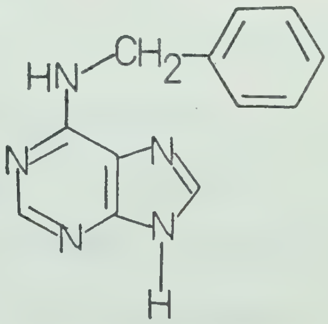
In the early 1950's Skoog and coworkers discovered that addition of coconut milk or yeast extracts to segments of tobacco callus tissue, grown on a synthetic medium, promoted new and continued cell division and growth. This property of causing increased cell division and growth in plants has subsequently been termed cytokinin activity and compounds that can produce this activity are called cytokinins.41a) This early work and the subsequent isolation, identification and synthesis of a number of purine derivatives that have cytokinin activity has been reviewed by Strong41b) and by Miller42) and will be but briefly covered here.

Skoog and coworkers were able to isolate a very small amount of material from yeast extracts that had high cytokinin activity when assayed on tobacco callus tissue. The properties of this material were consistent with a purine or purine derivative. These workers then turned to DNA, a rich source of purines, as a possible

source of cytokinin active compounds. Thus in 1955 Miller *et al*43) reported the isolation of a material from the DNA of herring sperm that was a highly active cytokinin. They named this material kinetin and subsequently identified it as 6-(2-furfurylamino)purine (14) and showed that synthetic kinetin had similar cytokinin activity to that of the isolated material.44) The initial isolation of kinetin was from a four year old preparation of DNA and freshly prepared samples of DNA were found to yield no kinetin. It was discovered however43) that autoclaved samples of fresh DNA yielded relatively large quantities of kinetin and Hall and deRopp45) later showed that kinetin is formed from the adenine and 2-deoxy-ribose residues of DNA during the autoclaving process.



14



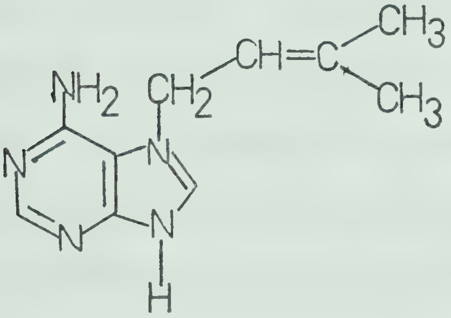
15

The isolation and structure determination of kinetin was quickly followed by reports of a number of syntheses of kinetin and of various kinetin analogues.[46,47,48] Of the many analogues of kinetin subsequently synthesized, generally only those that were 6-_N_-substituted-6-aminopurines showed significant cytokinin activity[41,42] and only a small number of these had an activity comparable to that of kinetin. Of particular interest was 6-_N_-benzylaminopurine (_15_) which has a cytokinin activity as great as or even greater than that of kinetin.[49]

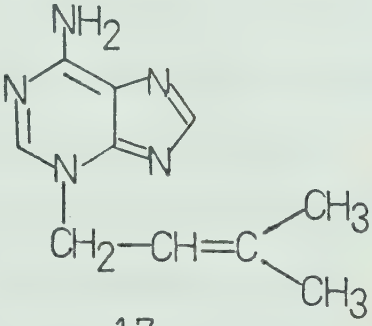
Although none of the synthetic cytokinins resembling kinetin (or kinetin itself) had been found to occur naturally, the occurrence of cytokinin activity in various plant extracts led both Strong[41b] and Miller[42] to speculate on the natural occurrence of kinetin like compounds as cell division factors. A subsequent study of the alkaloid triacanthine led to the discovery of a highly active, naturally occurring cytokinin.

Belikov _et al_[50] first reported the isolation of triacanthine from the young leaves of _Gleditsia triacanthos_ in 1954. In 1960 Leonard and Deyrup[51] erroneously reported the structure of triacanthine as 7-(3-methyl-2-butenyl)adenine (_16_). This structure assignment was later corrected to 3-(3-methyl-2-butenyl)-adenine (_17_) and this assignment was supported by Denayer _et al_[52] who reported unambiguous synthesis of

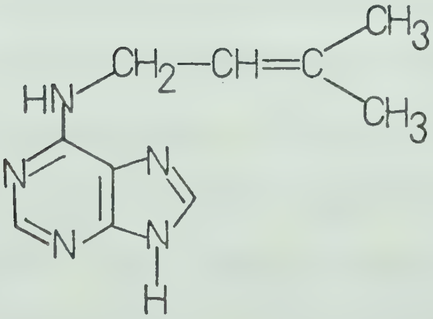
both 16 and 17. In 1962 Cavé *et al*[53)] noted that the concentration of triacanthine in the leaves of *G. triacanthos* was highest when the leaves were very young and decreased rapidly as the leaves aged.[50)] They found that triacanthine showed a very weak, but definite, cytokinin activity and speculated that triacanthine might play a similar role to kinetin in plants and might be involved in the mechanism of regulation of cell division. They also tested the *N*^{6}, 7, 8 and 9-(3-methyl-2-butenyl)adenine isomers of triacanthine



16



17



18

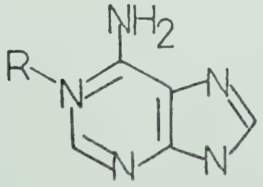
for cytokinin activity and found that, whereas the 7, 8 and 9-isomers were completely inactive, N^6-(3-methyl-2-butenyl)adenine (18) had an activity which was equal to or greater than that of kinetin. Beauchesne and Goutarel54) also found 18 to be a highly active cytokinin and in addition discovered that both N^6,N^6-bis-(3-methyl-2-butenyl)adenine and N^6,3-bis-(3-methyl-2-butenyl)adenine had activities that were only slightly reduced from that of 18.

In contrast to the work of Cavé *et al*,53) Rogozinska *et al*55) found that both naturally occurring and synthetic triacanthine were completely inactive when tested for cytokinin activity. However they found that when a sample of triacanthine was autoclaved prior to testing, significant cytokinin activity resulted. As autoclaving is a common procedure for the sterilization of samples prior to testing, they suggested that this was the reason for the cytokinin activity of triacanthine noted by Cavé *et al*. When an autoclaved sample of triacanthine was subjected to paper chromatography, although only one spot for triacanthine was observable on the chromatogram, elution of an area of the chromatogram adjacent to the triacanthine spot yielded an eluate that had a high cytokinin activity. Rogozinska *et al* suggested that this activity may result from rearrangement of triacanthine (17) to 18,

which they also found to be a highly active cytokinin, and they speculated upon the natural occurrence of 18 and suggested that triacanthine may act as a source of 18 in plants.

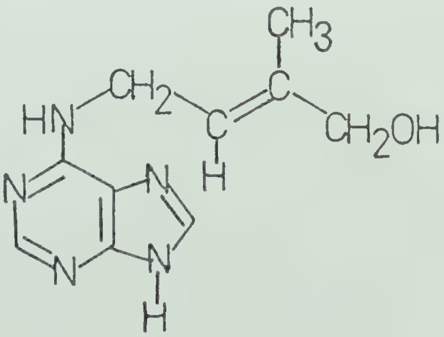
The isolation of a naturally occurring cytokinin from liquid cultures of *Corynebacterium fascians* was reported in 1966 by Klämbt *et al*[56)] and this was identified as 18 by Helgeson and Leonard.[57)]

The synthesis of 18 from 2'-deoxyadenosine (10) was reported by Leonard and Fujii[58)] in 1964. An intermediate in the synthesis was 1-(3-methyl-2-butenyl)-adenine (19). Also prepared were the analogous benzyl derivatives 1 and N^6-benzyladenine (20 and 15). Hamzi and Skoog[59)] tested the cytokinin activities of these compounds (15, 18, 19, 20) and found that the N^6-isomers (15 and 18) both had activities approximately 10 times greater than kinetin. However the 1-isomers (19 and 20) also showed relatively high activities. This was thought to be due to possible conversion of the 1 to the active N^6-isomers in the test system used and this was later shown to be the case.[60)]



19 $R = (CH_3)_2C{=}CH{-}CH_2{-}$

20 $R = C_6H_5CH_2{-}$



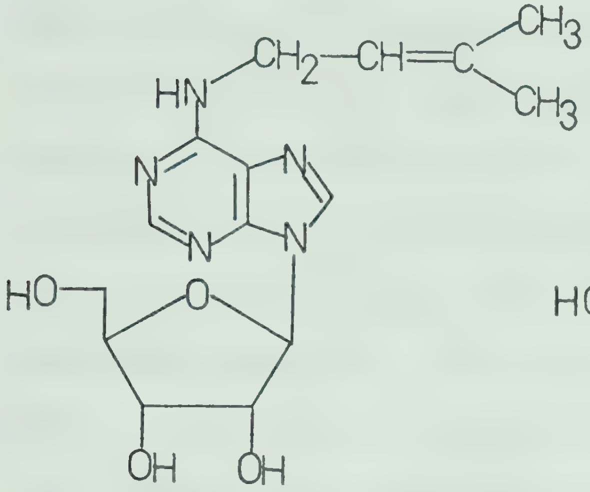
21

In 1963 Letham$^{61)}$ isolated a highly active cytokinin, as a crystalline picrate, from the immature seeds of a sweet corn variety of *Zea mays*. This material was named zeatin and it was thought to be similar to cytokinins previously isolated from young maize kernals$^{62)}$ and from plum fruitlets.$^{63)}$ The physical properties of zeatin again indicated that it was probably a 6-*N*-substituted-6-aminopurine derivative. Its structure was elucidated by Letham *et al*$^{64)}$ as being one geometric isomer of *N*^{6}-(4-hydroxy-3-methyl-2-butenyl)adenine, which is closely structurally related to **18**. The *trans*-isomer (**21**) was synthesized by Shaw *et al*$^{65)}$ and found to be identical to naturally occurring zeatin. Zeatin has been shown to have a cytokinin activity similar to that of **18** and greater than that of kinetin.$^{49)}$

B. THE OCCURRENCE OF CYTOKININS IN TRANSFER RIBONUCLEIC ACIDS.

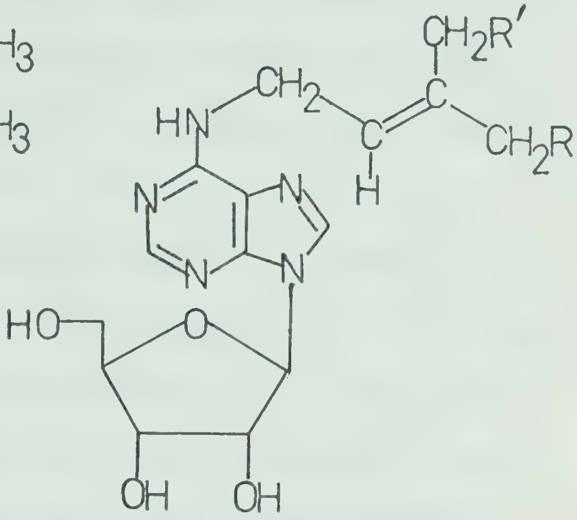
In 1966 Zachau *et al*[66)] reported the elucidation of the complete nucleotide sequences of two serine-specific tRNA's obtained from brewers yeast. The enzymatic hydrolysis of the tRNA's yielded, in each case, an unknown nucleoside which was situated adjacent to the 3'-end of the anticodon in the intact tRNA's. Biemann *et al*[67)] identified the structure of this nucleoside as $\underline{N}^6$-(3-methyl-2-butenyl)-9-β-D-ribofuranosyladenine (22) (iPA), the free base of which is identical to the highly active cytokinin 18 described above. Simultaneously, Hall and coworkers[68,69)] reported the isolation and identification of iPA from enzymatic hydrolysates of unfractionated yeast tRNA. Synthetic iPA was shown to be identical to the isolated material and it was noted that iPA had a significant cytokinin acitivity. Although the cytokinin activity of iPA (22) is appreciably reduced from that of the free base (18), it is still highly active and approaches the activity of kinetin in various systems.[49,69)] The amount of iPA isolated by Hall and coworkers[69)] was equivalent to 0.065 mole % of the total nucleoside residues in the crude tRNA and this correlates to only one tRNA molecule in about 20 containing an iPA residue. These workers were also able to detect similar amounts of iPA in crude tRNA preparations from calf

liver, human liver and chick embryos. They were however unable to detect any iPA in human liver or chick embryo rRNA.



22

IPA



23 R = H, R′ = OH

24 R = OH, R′ = H

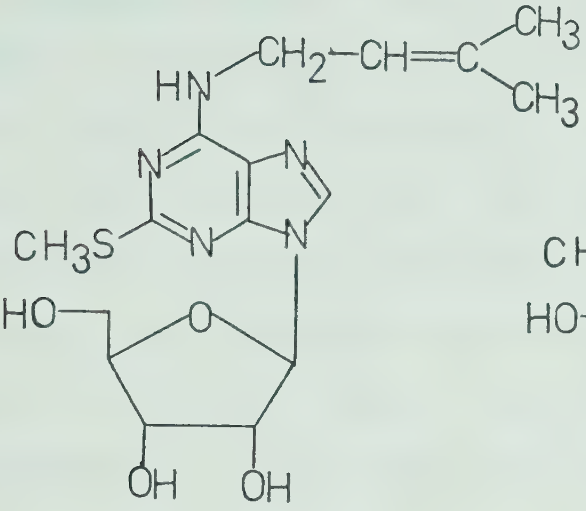
Following the initial discovery of the presence of iPA in some tRNA molecules, complete nucleotide sequence analysis of yeast tRNA$^{\text{Tyr}}$ 70) and of a single species of rat liver tRNA$^{\text{Ser}}$ 71) again revealed the presence of iPA molecules located adjacent to the 3'-end of the anticodon in each tRNA. IPA has also been detected in unfractionated tRNA's isolated from various plant and bacterial sources 72,73,74,75,76) as well as in certain specific tRNA's isolated from Escherichia coli 77) and yeast. 78)

Certain nucleosides which are closely related

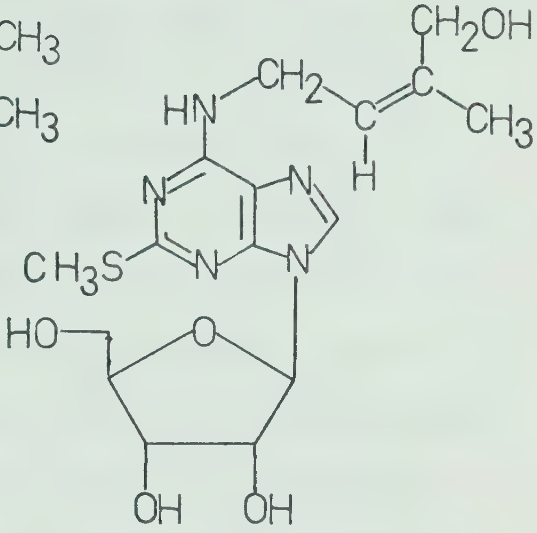
to iPA have also been found in tRNA molecules. In 1967 Hall et al[75)] reported the isolation of a cytokinin from the crude tRNA of spinach, sweet corn kernels and garden peas which he tentatively identified as N^6-(cis-4-hydroxy-3-methyl-2-butenyl)-9-β-D-ribofuranosyladenine (23). This structure assignment was later verified by Playtis and Leonard.[79)] The presence of 22 in unfractionated wheat germ tRNA[73,79)] as well as in E. coli, pea root and yeast tRNA's[74)] has also been reported. The free base of 22 is the geometric isomer of the widely occurring cytokinin zeatin (21), however the presence of the riboside of zeatin (24) in tRNA has been reported only once.[80)]

In 1968 Burrows et al[81)] reported the isolation of a cytokinin, having a similar activity to that of iPA,[76)] from the crude tRNA of E. coli. It was simultaneously found in E. coli tRNA^{Tyr} [82)] and was identified as 2-methylthio-N^6-(3-methyl-2-butenyl)-9-β-D-ribofuranosyladenine (25).[81,82)] This cytokinin has subsequently been found in tRNA preparations from a number of sources.[72,73,74,77,83)] A further cytokinin, that incorporates both of the modifications of iPA described above, has been isolated from unfractionated wheat germ tRNA and identified as 2-methylthio-N^6-(4-hydroxy-3-methyl-2-butenyl)-9-β-D-ribofuranosyladenine.[73)] This compound has also been found in tRNA hydrolysates from

the bacterium *Pseudomonas aeruginosa* and has been tentatively assigned the *cis* configuration (26).84)



25



26

In studies in which the position of the cytokinin in the tRNA molecule has been determined,70,71,74,85) only one cytokinin per tRNA molecule has been identified and it has been located adjacent to the 3'-end of the anticodon (see also reviews by Hall86,87) and by Skoog and Armstrong88)). Furthermore, it has been noted86,87,88,89) that these cytokinins occur only in those tRNA's which respond to codons beginning with U.

The potential function of iPA and its analogues in tRNA's has been investigated by a number of workers. Fittler and Hall90) selectively quaternized the iPA residue in yeast tRNA^{Ser} by treatment with iodine and

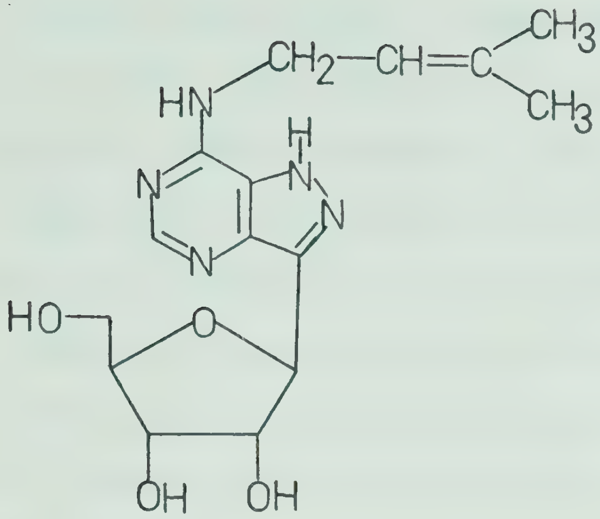
they found that the ability of the treated tRNA to bind to the mRNA-ribosome complex was significantly reduced. Gefter and Russell91) isolated three forms of suppressor $tRNA^{Tyr}$ which differed only in the degree to which the adenosine residue adjacent to the 3'-end of the anticodon was modified. They also found that the ability of these tRNA's to bind to the mRNA-ribosome complex depended on the degree of modification and concluded that the presence of cytokinins adjacent to the 3'-end of the anticodon in some tRNA's responding to codons beginning with U is necessary for the most efficient protein synthesis. However, experiments by Litwack and Peterkofsky92) did not support this conclusion.

C. THE BIOLOGICAL ACTIVITY OF CYTOKININS

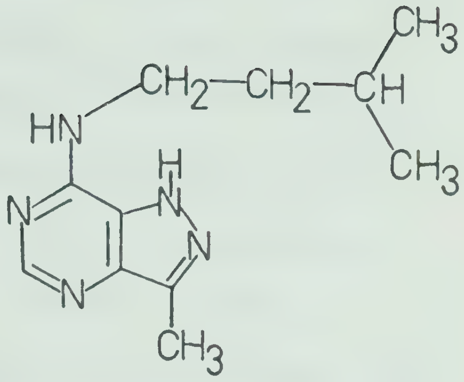
1. Cytokinin Activity

The cytokinin activity of a number of 6-N-substituted-6-aminopurines and related compounds has been described above and extensive reviews are available[41,42,87,88] (and references cited therein). The synthesis and activity of cytokinin related compounds containing varied 6-N-substituents[93,94,95,96,97] and modified purine bases[96,98,99] (see also reviews cited above) has received a great deal of attention and studies of the relationship between the structure and activity of cytokinins have appeared.[49,100]

As has been mentioned, the naturally occurring cytokinins existing as the free bases (18, 21) and as the 9-β-D-ribofuranosides (22, 23, 25) both show potent activity. Skoog et al[49] noted, however, that the ribosides generally had lower activity than the corresponding free bases and this observation was supported in an extensive study by Schmitz et al.[101] In 1971, Hecht et al[102] reported a number of pyrazolo-[4,3-d]pyrimidine derivatives, including 7-(3-methyl-2-butenylamino)-3-β-D-ribofuranosylpyrazalo[4,3-d]-pyrimidine (27), which are related to the cytokinins 18 and iPA (22). Compound 27, however, has a C-C glycosidic linkage which, in contrast to iPA, should



27



28

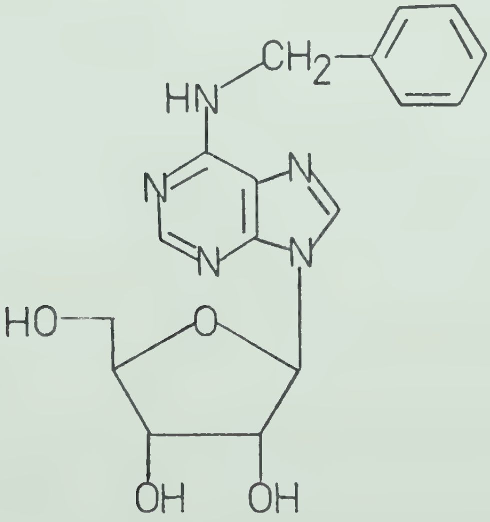
be stable to usual metabolic cleavage. Their results indicate that the presence of the ribose residue in a cytokinin is not required for it to show a high activity. A further interesting result of this work was the discovery that one of the compounds synthesized, 3-methyl-7-(3-methylbutylamino)pyrazalo[4,3-d]pyrimidine (28), was a potent cytokinin antagonist.[103,104]

2. Mammalian Inhibitory Activity

In 1967 Grace and coworkers[105] reported that iPA (22) is a potent growth inhibitor of a line of cells derived from human myelogenous leukemia and induced clinical remission in a case of acute leukemia during clinical trials.[106] IPA also showed potent inhibitory efects against Sarcoma-180 cells, but showed no sig-

nificant inhibition of other human tumor cells. IPA has since been reported to have inhibitory effects against various types of mammalian cell lines.107,108,109) The inhibitory activity of iPA in mammalian systems is in marked contrast to its cytokinin activity in plant systems, although stimulation of cell growth has been noted when iPA and some of its analogues were administered to a human leukemia cell line in extremely low concentration.92)

Fleysher and coworkers96,97,110) have reported the synthesis and growth inhibitory activity of a number of analogues of iPA having varying $\underline{N}^6$-substituents and modified bases. $\underline{N}^6$-benzyladenosine (29) had activity similar to that of iPA in a number of systems.96) Fleysher97) also found, as originally noted by Grace *et al*,105) that the presence of the sugar moiety was



29

necessary for significant mammalian inhibitory activity.

The mechanism of action of iPA is unknown at present. Some evidence indicates that iPA acts as an inhibitor of both DNA and RNA biosynthesis108) and it may interfer in pyrimidine metabolism.111) Rustum and Mihich112) however noted both inhibition and stimulation of DNA, RNA and protein biosynthesis in differing types of mammalian tissue. The metabolism of iPA in mammalian systems has been studied and it appears that iPA is active in the cell as its 5'-nucleotide.113,114) However the major degradative pathway of iPA is the rapid and irreversible cleavage of the sugar moiety to produce the inactive base 18.109,114) This degradation appears to be the major mechanism of resistance of cells to inhibition by iPA.113)

RESULTS AND DISCUSSION

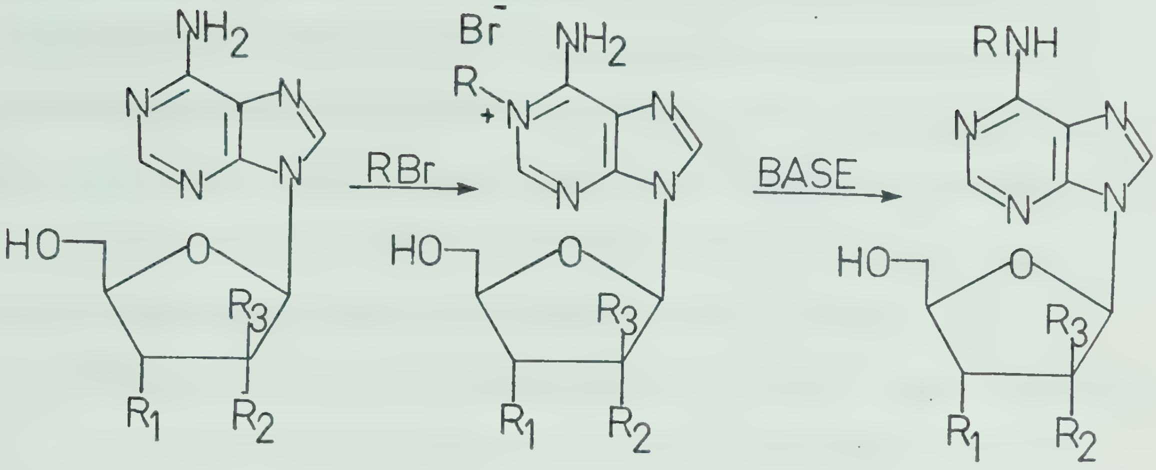
The work described in this section deals with the synthesis of a number of analogues of iPA. These were prepared for cytokinin, mammalian inhibition and other collaborative biochemical studies. Previous studies primarily concentrated on changes in the side chain or the base. In view of the necessity of the ribose residue for mammalian inhibitory activity and also of the rapid inactivation of iPA by enzymatic cleavage of the ribose residue (see Introduction), this work concentrated mainly on iPA analogues which are modified in the sugar residue. Preparation of the formycin analogue of iPA (42), which has a carbon-carbon glycosyl linkage that is presumably stable to usual metabolic cleavage, was of interest for the same reasons. Also undertaken was the synthesis of a similar series of the biologically analogous but more chemically stable benzyl analogues of iPA. Finally iPA analogues derived from 6-mercaptopurine riboside and 6-thioguanosine where chosen in view of the marked activity of 6-mercaptopurine and its derivatives.115)

A. SYNTHESIS OF ANALOGUES OF IPA

$\underline{N}^6$-(3-Methyl-2-butenyl)adenosine (iPA) (22) was first synthesized by Leonard and coworkers 60,116) by $\underline{N}^1$-alkylation of adenosine with 3-methyl-2-butenyl bromide followed by Dimroth rearrangement 117) in base to the $\underline{N}^6$-isomer (see Scheme I). Overall yields of 38% 116) and 42.5% 69) have been reported for this reaction sequence. However, we were unable to obtain yields greater than 20-30% following the same procedure. We therefore investigated means to improve this method of synthesis.

In accordance with the observations of Leonard *et al* 60) and of Martin and Reese, 118) we also observed that the alkylation step proceeded to about 50-60% and then ceased. Shimizu and Miyaki 114) have reported the HBr catalyzed migration of 3-methyl-2-butenyl and benzyl groups, presumably by reversal of the alkylation reaction by bromide ion followed by realkylation. In order to circumvent the possibility of such reversal reactions during the alkylation process, molecular sieves were added to the reaction mixture as a presumed absorbent of hydrogen bromide. This rationalization was later shown to be incorrect, however the alkylation was observed to proceed to 80-90% using this modification and crystalline yields of iPA approaching 58% were obtained. This method was therefore used for the alkyla-

SCHEME I



6 $R_1=R_2=OH$, $R_3=H$

30 $R_1=R_3=OH$, $R_2=H$

10 $R_1=OH$, $R_2=R_3=H$

31 $R_2=OH$, $R_1=R_3=H$

$iP=(CH_3)_2C{=}CH{-}CH_2{-}$

$Bn=C_6H_5CH_2{-}$

22 $R=iP$, $R_1=R_2=OH$, $R_3=H$

29 $R=Bn$, $R_1=R_2=OH$, $R_3=H$

32 $R=iP$, $R_1=R_3=OH$, $R_2=H$

33 $R=Bn$, $R_1=R_3=OH$, $R_2=H$

34 $R=iP$, $R_1=OH$, $R_2=R_3=H$

35 $R=Bn$, $R_1=OH$, $R_2=R_3=H$

36 $R=Bn$, $R_2=OH$, $R_1=R_3=H$

tion of 2'-deoxyadenosine (10) and 9-β-D-arabinofuranosyladenosine (30) with 3-methyl-2-butenyl bromide, as described below.

It was later discovered that the addition of one equivalent of LiBr to the alkylation reaction caused no observable change in the degree of alkylation. Also a sample of the N^1-alkylated product, which was isolated

by preparative thin layer chromatography (tlc), gave no observable reversal to adenosine when treated with 2 equivalents of HBr in DMF. It was also noted that the reaction became appreciably acidic, even in the presence of molecular sieves. When $BaCO_3$ was added to the reaction mixture, no acidity was observed and the alkylation appeared essentially quantitative. Using this procedure yields of iPA approaching 74% have been obtained.

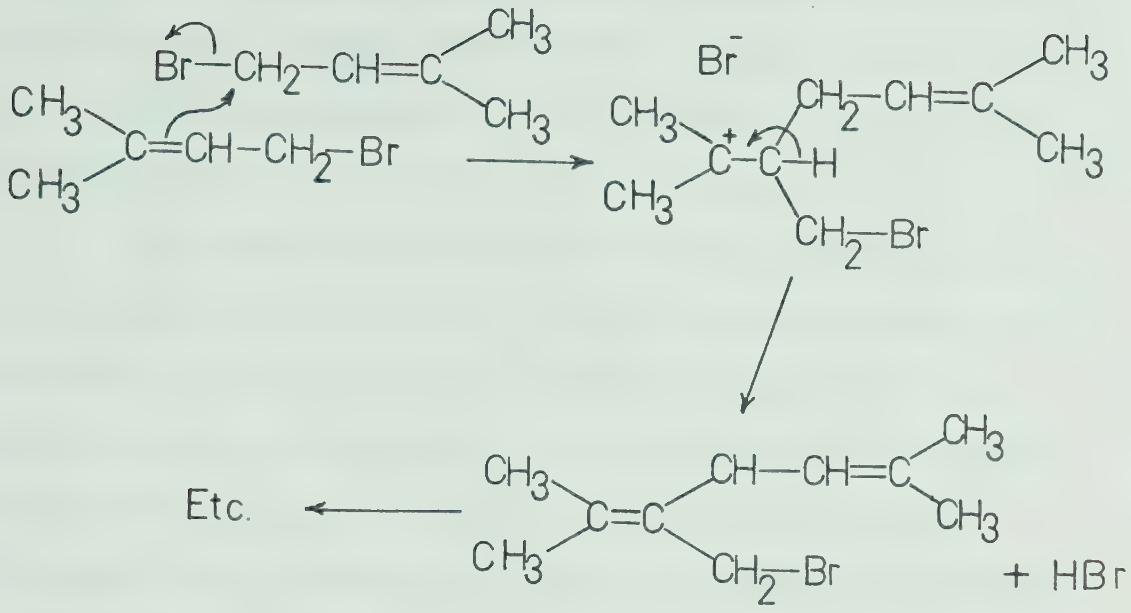
It appears therefore that HBr, produced during the reaction, inhibits the alkylation process. If sufficient HBr is generated to protonate about 50% of the adenosine molecules by the time that alkylation has occurred to 50%, the resulting adenosine $\underline{N}^1$-hydrobromide would be inaccessible to alkylation. Addition of $BaCO_3$ as an efficient nonnucleophilic acid acceptor therefore prevents formation of the hydrobromide salt and allows the reaction to proceed to completion. Use of molecular sieves as acid acceptor was less efficient and resulted in only moderately increased alkylation yields.

It was also later discovered that samples of iPA synthesized by the alkylation-rearrangement procedure invariably contained a small amount of material (which was not completely removed by recrystallization) which migrated faster than iPA in chromatographic systems and had a molecular weight = 403 (mass spectroscopy). This

corresponds to an iPA molecule with a hydrogen atom substituted by a second 3-methyl-2-butenyl group (335 - 1 + 69). A small amount of this material was isolated by column chromatography along with a trace amount of a second impurity that had mol. wt. = 471 (iPA substituted by two 3-methyl-2-butenyl groups). The ultraviolet spectra of these two impurities were identical to that of iPA. The nmr spectrum of the mol. wt. = 403 material was not readily interpretable, but showed a complex pattern of resonances in the $\delta 1.0$ to 2.0 range. Hydrogenation of this material over Raney Nickel yielded a compound that had mol. wt. = 407, indicating the presence of two olefinic bonds. No other structural information on this material was obtained. However, if 3-methyl-2-butenyl bromide molecules are assumed to condense during the alkylation procedure, for example as outlined in Scheme II, this would account for production of HBr during the reaction. Alkylation of adenosine by the resulting condensed alkenyl bromide products would give rise to the high molecular weight impurities observed.

The earlier reports on the preparation of iPA69,116) both used refluxing aqueous base to effect the Dimroth rearrangement of the $\underline{N}^1$- to the $\underline{N}^6$-alkylated product (22). Martin and Reese118) later reported a much milder technique involving treatment of the $\underline{N}^1$-alkylated product with 50% Me_2NH-MeOH at room temperature. This method

SCHEME II



proved to be very effective and resulted in complete rearrangement in from 1 to 4 hours for a number of the compounds studied.

A further note on iPA *per se* concerns its optical rotation. Various samples prepared by the procedure of Leonard *et al*,[60,116)] Martin and Reese[118)] and our modification gave $[\alpha]_D$ values near -70° and $[\alpha]_{546}$ near -85° in ethanol. These values are significantly different from the reported values of $[\alpha]_D^{28}$ -103° (c 0.14, EtOH)[60)] and $[\alpha]_{546}^{25}$ -97° (c 0.07, EtOH).[69)] A carefully purified sample of iPA which had correct elemental analyses and no higher molecular weight impurities had $[\alpha]_D^{23}$ -71.5° (c 0.14, abs. EtOH), $[\alpha]_D^{23}$ -70.0° (c 0.07, abs. EtOH), $[\alpha]_{546}^{23}$ -85° (c 0.14, abs.

EtOH) and $[\alpha]_{546}^{23}$ -85.8° (c 0.07, abs. EtOH). These values are consistent with those obtained in 95% EtOH and also with values obtained on a "drug" sample of iPA (kindly provided by Dr. G.B. Chleda, Roswell Park Memorial Institute, Buffalo, N.Y.) within ± 1.5°.

Synthesis of $\underline{N}^6$-(3-methyl-2-butenyl)-9-β-D-arabinofuranosyladenine (32) from 30 was analogous to the preparation of iPA. Using molecular sieves as the acid acceptor, compound 32 was obtained in 54% crystalline yield. Removal of the high molecular weight impurity was achieved by recrystallization from MeOH-iPrOH. After this work was completed, a patent appeared describing 32 prepared by a base sugar coupling procedure.[120)] The preparation of $\underline{N}^6$-(3-methyl-2-butenyl)-2'-deoxyadenosine (34) was also carried out similarly, from 10, but greater care was necessary in order to avoid cleavage of the relatively acid labile glycosidic bond. Martin and Reese[118)] and Brookes et al[121)] have previously obtained only the alkylated adenine aglycone salts when 2'-deoxyadenosine (10) was treated with 3-methyl-2-butenyl bromide and benzyl bromide respectively. Leonard et al[95)] have reported the synthesis of 34, which was characterized as a hygroscopic solid hydrate with mp 48-49°C and $[\alpha]_D^{25}$ -9.9° (c 0.8, EtOH). In this case crystalline 34, obtained after a protracted purification procedure, had mp 106-109°C and $[\alpha]_D^{28}$ -19.5° (c 1, MeOH).

Sivadjian et al[122] have reported the enzymatic transfer of 2-deoxy-D-erythro-pentose (2-deoxyribose) to N6-(3-methyl-2-butenyl)adenine (18) and 6-benzylaminopurine (15) to presumably give the 2'-deoxy-nucleosides (34 and 35), but no characterization of products was given.

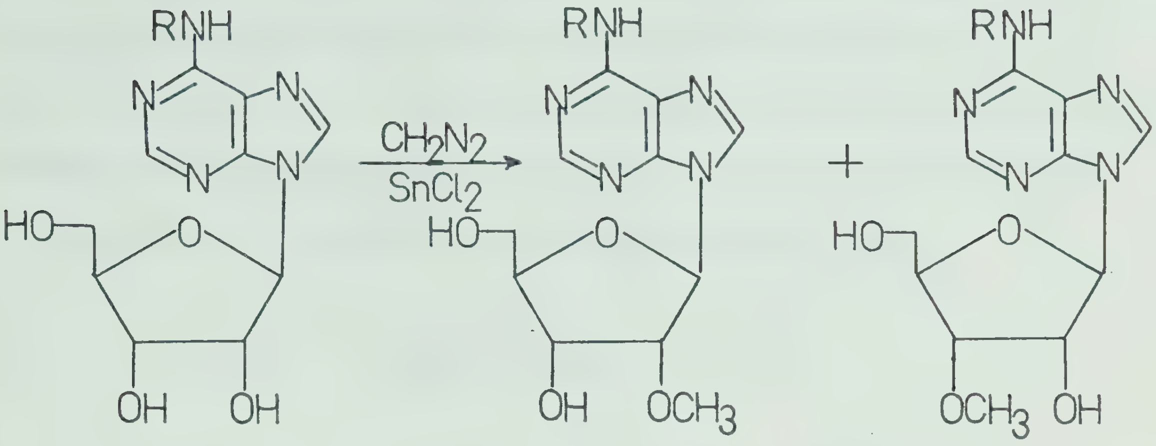
The preparation of the N6-benzyl analogues (29, 33, 35 and 36) was essentially analogous to that of iPA (see Scheme I), involving the usual N1-alkylation followed by rearrangement. In this case, however, the alkylation proceeds readily to ~90% in the absence of any acid acceptor. The benzylations of adenosine (6) and 9-β-D-arabinofuranosyladenine (30) were effected in a manner similar to that described by Fleysher et al[96] for the benzylation of 6. Use of the milder Me_2NH-MeOH rearrangement procedure gave N6-benzyladenosine (29) and N6-benzyl-9-β-D-arabinofuranosyladenine (33) in 67% and 69% crystalline yields, respectively. The benzylation of 10 proceeded similarly to yield crystalline N6-benzyl-2'-deoxyadenosine (35) after column chromatographic purification. 3'-Deoxyadenosine (Cordycepin) (31) was prepared according to the method developed by Robins et al[123] in this laboratory. Benzylation of 31 proceeded smoothly to yield, after rearrangement, N6-benzyl-3'-deoxyadenosine (36).

The 2'-O- and 3'-O-methyl derivatives of 22 and 29 were interesting in view of the reported inertness

of certain 2'-O-methyl nucleosides to the action of hydrolase and phosphorylase enzymes,[124] enzyme systems that catalyze cleavage of the glycosyl bond. Also, 3'-O-methyluridine is apparently inert to the action of uridine phosphorylase.[125]

Initially synthesis of these derivatives from 2'-O- and 3'-O-methyladenosine was attempted, following the usual alkylation-rearrangement procedure. However, although the reactions appeared to proceed normally, purification was difficult and crystalline products were not obtained. A more convenient procedure was the direct methylation of 22 and 29 using the conditions described by Robins and Naik.[126,127] Thus 22 and 29 were quantitatively monomethylated by diazomethane in the presence of stannous chloride to yield mixtures of the 2'-O- and 3'-O-methyl isomers (37 and 38, 39 and 40, respectively) (see Scheme III). The 2'-O- and 3'-O-methyl isomers were obtained in ratios of about 40:60, as was observed with adenosine.[126] The 3'-isomer, 38, readily crystallized from the mixture of 37 and 38 and the remaining material was separated by column chromatography on Dowex 1-X2(OH^-) resin.[128] The 2'-isomer, 37, did not crystallize. It was obtained in an analytically pure state after a further purification procedure followed by freeze drying. The mixture of 39 and 40 was separated directly by column chromatography[128] and

SCHEME III



22 R = iP

29 R = Bn

37 R = iP

39 R = Bn

38 R = iP

40 R = Bn

iP = $(CH_3)_2C{=}CH{-}CH_2{-}$

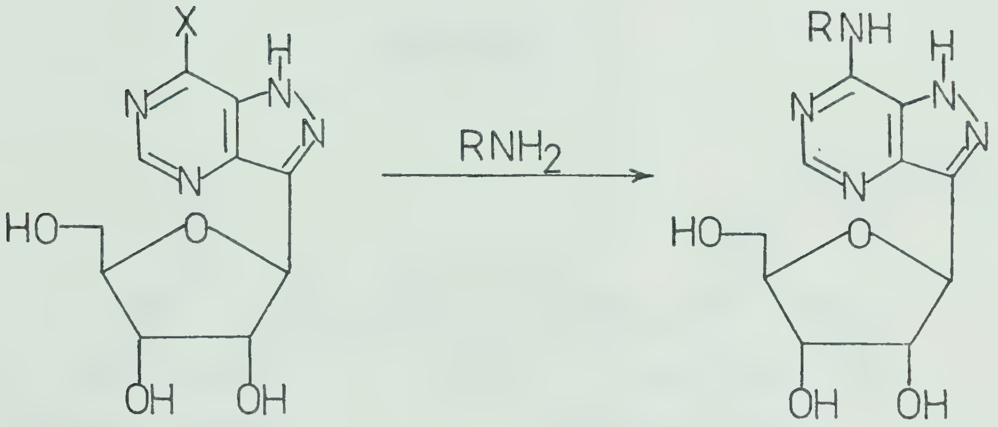
Bn = $C_6H_5CH_2{-}$

the 3'-isomer (40) crystallized readily. Again the 2'-isomer did not crystallize directly and was converted to its crystalline hydrochloride salt.

Hecht *et al*102) reported the synthesis of 7-*N*-(3-methyl-2-butenyl)amino-3-β-*D*-ribofuranosylpyrazolo-[4,3-*d*]pyrimidine [*N*^{7}-(3-methyl-2-butenyl)formycin] (42) by alkylation of formycin followed by rearrangement to the *N*^{7}-isomer (an analogous reaction sequence to that used for the preparation of iPA, Scheme I). These authors reported that 42 was obtained in "low yield" and record mp 120-122°C. In previous studies

of this reaction we had found that at least five products (spots visible under uv light on tlc) are present in the alkylation solution in the presence or absence of molecular sieves. It is one of the minor intensity spots which disappears upon rearrangements in base and a minor spot corresponding to 42 appears.

SCHEME IV



41 X = Cl

44 X = SCH_3

42 R = $(CH_3)_2C{=}CH{-}CH_2{-}$

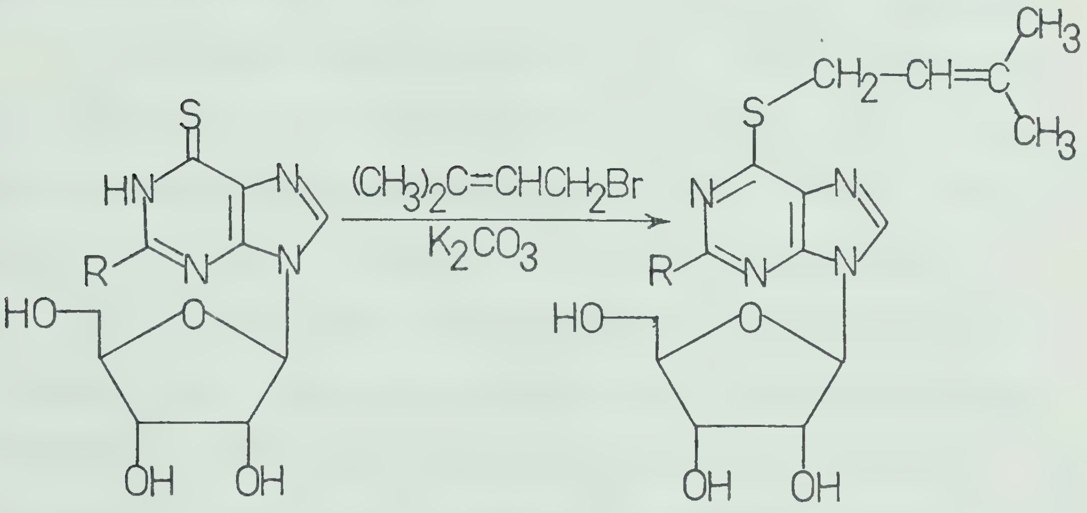
43 R = $C_6H_5CH_2{-}$

Compound 42 was readily prepared, in 64% yield, by treatment of 7-chloro-3-β-*D*-ribofuranosylpyrazolo[4,3-*d*]pyrimidine (41)[129] with 3-methyl-2-butenylamine (Scheme IV) and had mp 193.5-195°. Analogous reaction using benzylamine gave *N*^{7}-benzylformycin (43) in 70% yield after purification by preparative tlc. This material was obtained in crystalline form as its hydro-

chloride salt. Earlier attempts to utilize the 7-methylthio derivative of formycin (44) in this process proved far less successful.

Sulphur alkylation of 6-mercaptopurine riboside (45) and 6-thioguanosine (46) proceeded rapidly to completion in dry dimethylformamide in the presence of anhydrous potassium carbonate[130)] to give the corresponding 3-methyl-2-butenyl thionucleosides 47 and 48,

SCHEME V



45 R = H

46 R = NH_2

47 R = H

48 R = NH_2

respectively (Scheme V). Attempts to crystallize these products resulted in gel formation and both were obtained as analytically pure amorphous solids by precipitation into Skellysolve 'B'.

B. MASS SPECTRAL AND NMR OBSERVATIONS

The mass spectroscopy of cytokinins[131,132] and modified nucleosides found in tRNA[73,133,134] is a technique that has received a great deal of attention, and is extremely useful in the identification of such compounds isolated in small amounts from natural sources. Mass spectroscopy was also a valuable tool for the characterization of the cytokinin analogues synthesized in this study and was particularly useful for distinguishing the 2'-O- and 3'-O-methyl isomers.

McClosky and coworkers[135,136] have shown that the mass spectra of nucleosides generally show a series of characteristic fragmentations. Some of the fragment ions of the 3-methyl-2-butenyl analogues and of the benzyl analogues are summarized in tables I and II, respectively. The more structurally significant ions consist of the heterocyclic base (B) plus various portions of the sugar skeleton. A second series arises by fragmentation of the alkenyl side chain either from the molecular ion (M) or, more abundantly, from the ion corresponding to the free base (BH) (see Scheme VI). Loss of $C_{5'}$ as formaldehyde with concomitant proton transfer to the base gives a low intensity ion (c) at m/e M-30. This is characteristic of nucleosides having an unsubstituted 5'-hydroxyl group and is observed in

SCHEME VI

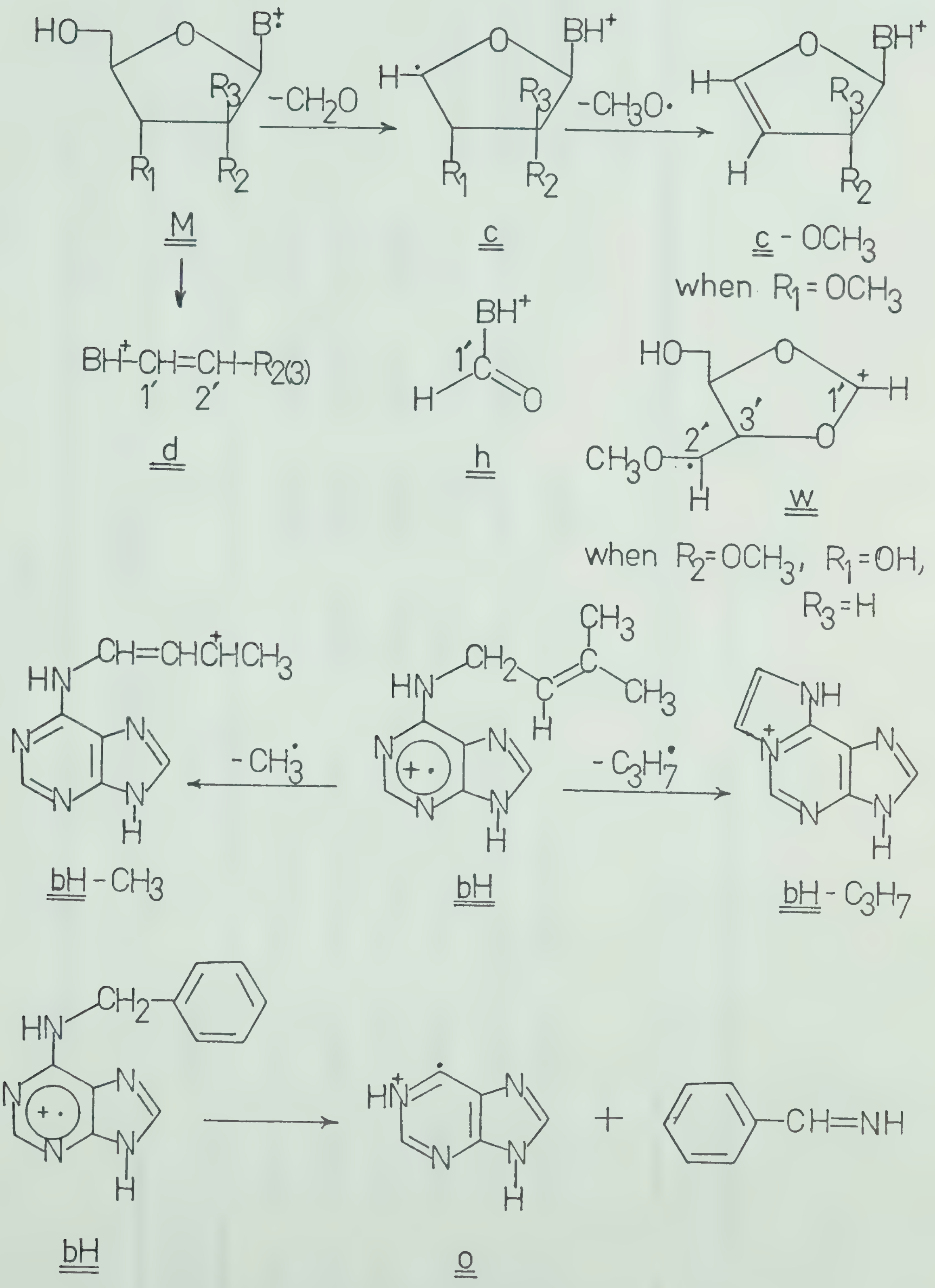

- CH2O
- CH3O·
M
c
c - OCH3
when R1 = OCH3
BH+-CH=CH-R2(3)
d
h
w
when R2 = OCH3, R1 = OH, R3 = H
- CH3·
- C3H7·
bH - CH3
bH
bH - C3H7
bH
o
CH=NH

TABLE I

Characteristic Mass Spectral Ions of 3-Methyl-2-butenyl Analogues

m/e (relative intensity (%))

Comp[D]	M	M-CH_3	c	M-C_3H_7	d	h	BH	BH-C_3H_7	Others
22	335(71)	320(9.3)	305(2.3)	292(36)	246(18)	232(27)	203(69)	160(100)	
32	335(36)	320(41)	305(0.8)	292(12)	246(12)	232(33)	203(59)	160(100)	
34	319(29)	304(1.3)	289(2.7)	276(2.1)	230(4.2)	232(24)	203(50)	160(100)	
37	349(100)	334(7.6)	319(11)	306(15)	260(56)	232(13)1	203(68)	160(91)	(w), 146(10)
38	349(68)	334(9.0)	319(2.8)	306(13)	246(13)	232(16)	203(71)	160(100)	(c-OCH_3), 288(18)
42[a]	335(8.8)	320(2.3)	305(0.2)	292(1.8)	246(8.1)	232(42)	-	-	(d-C_5H_8), 178(9.1) (h-C_5H_8), 164(45)

---

[a] The base peak of this spectrum was at m/e 41.

TABLE II

Characteristic Mass Spectral Ions of Benzyl Analogues

m/e (relative intensity (%))

Comp[D]	M	c	d	h	BH	o	Others
29	357(28)	327(3.0)	268(15)	254(29)	225(100)	120(18)	
33	357(35)	327(2.3)	268(14)	254(54)	225(100)	120(11)	
35	341(27)	311(4.2)	252(12)	254(32)	225(100)	120(17)	
36	341(18)	311(3.8)	268(39)	254(25)	225(100)	120(16)	
39[a]	371(35)	341(10)	282(40)	254(21)	225(100)	120(14)	(w), 146(15)
40[b]	371(11)	341(1.2)	268(4.6)	254(15)	225(10)	120(15)	(c-OCH_3), 310(4.1)
43[b]	357(9.5)	-	268(10)	254(53)	225(1.1)	-	

[a] The spectrum of this compound was obtained on the hydrochloride salt, which vapourizes in the spectrometer as the free nucleoside. [b] The base peak of this spectrum was at m/e 91.

all of the present compounds. Sugar cleavage to ion d, which contains $C_{1'}$, $C_{2'}$ and the 2'-substituent, gives rise to a peak at m/e B+44 in compounds having an unsubstituted 2'-hydroxyl group. However, in the 2'-O-methyl derivatives 37 and 39 this ion appears at m/e B+58 and in the 2'-deoxy derivatives 34 and 35 at m/e B+28, thus indicating the nature of the substituent at the 2'-position. Ion w, which is also characteristic of 2'-O-methylation,136) occurs at m/e 146 in 37 and 39 and is absent in the spectra of the 3'-O-methyl compounds 38 and 40. An ion that occurred exclusively in the spectra of the 3'-O-methyl derivatives, and which may be characteristic of 3'-O-methylation, had m/e M-61. Accurate mass determination is consistent with the molecular formula $c\text{-}OCH_3$ and this ion could readily arise by loss of a methoxyl radical from ion c (see Scheme VI). Ion h is normally a high intensity ion and occurs at m/e B+30. It consists of the protonated base plus $C_{1'}$, $H_{1'}$ and the 4'-heteroatom and was observed in all of the compounds studied. Glycosidic bond cleavage, accompanied by transfer of up to two protons to the base, gives rise to ions B, BH and BH_2. These ions are characteristic of the base and its substituents, and the most intense of these, ion BH, occurs at m/e 203 in the spectra of the N^6-(3-methyl-2-butenyl) derivatives (22, 32, 34. 37 and 38)

and at m/e 225 for the *N*^{6}-benzyl derivatives (29, 33, 35, 36, 39 and 40).

Fragmentation of the 3-methyl-2-butenyl side chain has been studied previously by Shannon and Letham131) and gives rise to a characteristic series of ions. The *N*^{6}-(3-methyl-2-butenyl-nucleosides (22, 32, 34, 37 and 38) all show this series of ions which in iPA (22) occur at m/e 188 (**BH**-CH_3, 58%), 160 (**BH**-C_3H_7, 100%), 148 (**BH**-C_4H_7, 16%), 135 (**BH**-C_5H_8, 57%) and 41 ($C_3H_5^+$, 32%). This is in addition to ions arising directly from side chain fragmentation in the molecular ion at m/e **M**-15 (**M**-CH_3) and **M**-43 (**M**-C_3H_7) (see Scheme VI and Table I).

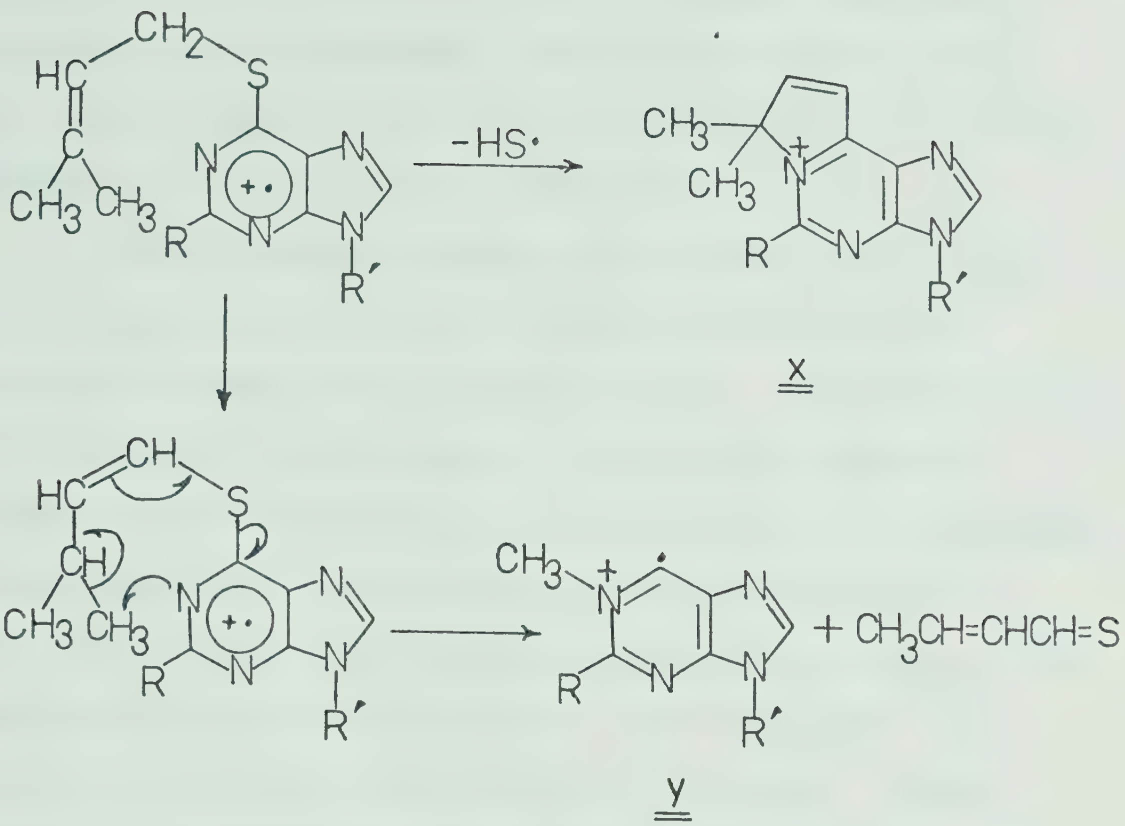
The mass spectra of the benzyl derivatives (29, 33, 35, 36, 39 and 40) also have a series of characteristic ions, which are analogous to those found with 6-benzylaminopurine (15).131) For example the spectrum of *N*^{6}-benzyladenosine (29) has peaks at m/e 148 (**BH**-C_6H_5, 6.6%), 106 ($C_6H_5CH{=}NH_2^+$, 46%) and 91 ($C_7H_7^+$, tropyllium ion, 51%). The benzyl derivatives also give rise to ion **o**136) by extrusion of benzylamine from ion **BH** with accompanying transfer of a hydrogen to the purine ring (see Scheme VI and Table II). The formycin derivative, 43, has the tropyllium ion as its mass spectral base peak, m/e 91 ($C_7H_7^+$, 100%).

The spectrum of *N*^{7}-(3-methyl-2-butenyl)formycin (42) has previously been discussed by Hecht.137) Spectra

of both 42 and 43 have high intensities of ion h and very low intensities of ion BH due to the stability of the C-C glycosidic bond.[138] This stabilty is also evidenced by significant ions at d-C_5H_8 and h-C_5H_8, in the spectrum of 42, in which side chain loss from these "glycoside" linked fragments (d and h) occurs. An ion at m/e M-36 (M-$2H_2O$) is present in the spectrum of 43 but not in 42 and thus, this loss is not general for formycin derivatives.[138]

The mass spectra of the 6-thio analogues 47 and 48 are similar to that of 6-(3-methyl-2-butenyl)thiopurine.[137] Extrusion of an $HS^{\bullet}$ radical to give ion x occurs from both the molecular ion and the BH ion (see Scheme VII). Ion y, which corresponds to formal loss of C_4H_6S from BH, is apparently significant only after loss of the sugar. Hecht[137] has suggested this ion as the 6-methylpurine isomer which would involve a rather complex single step or multiple step decomposition. He quotes evidence for a single step fragmentation in the observation of a peak at m/e 85 (C_4H_6S), which we also observe as a low intensity ion. A cyclic process evolving the 1-methyl isomer (ion y) plus thiocrotonaldehyde represents a plausible pathway for the formation of this ion (see Scheme VII). Remaining significant ions correspond to the usual sugar and side chain fragmentation patterns. For example, the spectrum

SCHEME VII



	R	R'	x,m/e(rel.int.)	y,m/e(rel.int.)
47	H	$C_5H_9O_4$	319 (5.7%)	
	H	H	187 (84%)	134 (38%)
48	NH_2	$C_5H_9O_4$	334 (12%)	
	NH_2	H	202 (100%)	149 (21%)

of 47 has peaks at m/e 352 (M, 13%), 263 (d, 2.4%), 249 (h, 1.8%), 220 (BH, 22%), 152 (BH-C_5H_8, 4.4%) and 41 ($C_3H_5^+$, 100%).

Nuclear magnetic resonance (nmr) spectroscopy

was also useful for characterization of the compounds prepared and clearly indicated the major structural features of each compound. The ^1H nmr spectra were all run in $(CD_3)_2SO-D_2O$ mixed solvent, thus eliminating peaks due to exchangeable hydrogens.

The 3-methyl-2-butenyl derivatives (22, 32, 34, 37, 38, 42, 47 and 48) all showed a characteristic series of peaks due to the side chain. The methyl groups both absorbed in the range δ 1.68-1.75 and appeared either as a singlet or a pair of singlets. The methylene group appeared in the range δ 3.95-4.15, although it was often obscured by resonances due to $H_{3'}$ or $H_{4'}$, and the methine group appeared as a broad triplet in the range δ 5.25-5.45 with a coupling constant ~ 6-7 Hz. The benzyl derivatives (29, 33, 35, 36, 39, 40 and 43) also gave characteristic peaks. Thus the aromatic hydrogens of the phenyl ring invariably appeared as a broad singlet or multiplet at δ~ 7.34 and the methylene group gave a broad singlet at δ~ 4.78. The singlets due to H_2 and H_8 of the purine base, and also of H_5 in the formycin derivatives, appeared in the range δ 8.20-8.45 and were generally separated by ~ 0.1-0.15 ppm. The only exceptions to this were the 6-thiopurine derivatives (47 and 48) and the 9-β-D-arabinofuranosyl derivatives (32 and 33). In 47 H_2 and H_8 appear at δ 8.67 and δ 8.73, respectively, whereas in 48, H_8 was at δ 8.17. In 32

$\underline{H}_2$ and $\underline{H}_8$ appear as a single peak at δ 8.20 and in 33 they also appear quite close together at δ 8.27 and δ 8.33.

The methylated derivatives (37, 38, 39 and 40) showed a characteristic singlet for the O-methyl group. In both of the 2'-isomers (37 and 39) this appeared at δ 3.33, whereas in the 3'-isomers it was shifted slightly downfield (δ 3.43 for 38 and δ 3.47 for 40). This downfield shift of the 3'-O-methyl group relative to the 2'-isomer has been observed previously in the spectra of a number of 2'- and 3'-O-methyl purine nucleosides.127) The deoxy nucleosides (34, 35 and 36) showed a pair of multiplets at relatively high field for the 2'- or 3'-methylene hydrogens (see Table III). Also, the 2'-deoxy derivatives (34 and 35) were readily distinguished from the 3'-isomer (36) by the splitting of the $\underline{H}_{1'}$ signal. In 34 and 35 $\underline{H}_{1'}$ appeared as a triplet whereas in 36 it was a doublet (see Table IV).

The chemical shifts of the sugar hydrogens and the coupling constants for $\underline{H}_{1'}$ are recorded in Tables III and IV, respectively. Some trends are apparent on inspection of this data. Most notable is the great similarity in the spectra of the sugar hydrogens for pairs of compounds differing only in the base substituent (3-methyl-2-butenyl or benzyl). This indicates that the nature of the base substituent, in these cases, does

TABLE III

Chemical Shifts of Sugar Hydrogens

Compound	Structural Feature	Shift of Hydrogen (δ in ppm)				
		$H_{1'}$	$H_{2'(2'')}$	$H_{3'(3'')}$	$H_{4'}$	$H_{5',5''}$
22	RIBO	5.94	4.58	~4.15	~4.15	3.63
29		5.93	4.61	4.21	4.08	3.60
32	ARABINO	6.29	~4.15	~4.15	~3.7	~3.7
33		6.36	~4.2	~4.2	3.86	3.72
34	2'-DEOXY	6.36	2.33 2.75	4.46	3.95	3.63
35		6.38	2.30 2.70	4.45	3.94	3.62
36	3'-DEOXY	5.90	4.61	1.95 2.29	4.40	3.60
37	2'-O-METHYL	6.03	~4.4	~4.4	4.02	3.70
39		6.07	4.42	4.42	4.02	3.70
38	3'-O-METHYL	5.92	4.77	4.12	3.88	3.64
40		5.92	4.79	4.12	3.91	3.68
42	FORMYCIN	4.98	4.46	4.13	3.97	3.62
43		5.03	4.53	4.15	3.97	3.65
47	6-THIO	6.02	4.63	4.24	4.00	3.69
48		5.82	4.33	4.17	3.95	3.64

TABLE IV

Coupling Constants of $C_{1'}$ Hydrogens

3-Methyl-2-butenyl Derivatives			Benzyl Derivatives		
Compound	Multiplicity[a)]	$J_{1'-2'(2'')}$ (Hz±0.2)	Compound	Multiplicity[a)]	$J_{1'-2'(2'')}$ (Hz±0.2)
22	d	6.1	29	d	6.2
32	d	4.2	33	d	4.2
34	t	7.0	35	t	7.0
37	d	4.8	36	d	3.0
38	d	6.3	39	d	4.8
42	d	7.0	40	d	6.0
47	d	5.3	43	d	7.0
48	d	6.1			

[a] d = doublet, t = triplet

not have any significant effect on the chemical environment or conformation of the sugar residue. The position of the $\underline{H}_{1'}$ resonance is dependant on both the nature of the base and of the neighbouring 2'-substituent. Thus in those adenine derivatives having a 2'-hydroxyl group cis to the 1'-hydrogen (22, 29, 36, 38 and 40), the $\underline{H}_{1'}$ signal appears in the narrow range of δ 5.90-5.94. However in the arabino derivatives (32 and 33) and the 2'-deoxy derivatives (34 and 35), which now have a hydrogen at the 2'-position cis to the 1'-hydrogen, the $\underline{H}_{1'}$ signal is shifted downfield by ~ 0.35-0.45 ppm. This shift is presumably due to the absence of the shielding effect of the neighbouring cis hydroxyl group.139,140) Distinct, although much smaller (0.1-0.15 ppm), downfield shifts are also observed in the spectra of the 2'-O-methyl derivatives (37 and 39). The presence of the C-C glycosyl bond in the formycin derivatives (42 and 43) is characterized by the marked upfield shift observed for the $\underline{H}_{1'}$ signal.

The $\underline{H}_{2'}$ signal generally appears at about δ 4.5-4.6, except in the case of the 2'-deoxy derivatives discussed above. The most notable exceptions are the arabino derivatives, 32 and 33, where the 2'-hydrogen, which now has the opposite configuration, gives a signal that is shifted upfield by ~ 0.4 ppm.

Slight upfield shifts are also observed for the 2'-O-methyl derivatives (37 and 39) and slight downfield shifts for the 3'-O-methylisomers (38 and 40). Marked downfield shifts of the $H_{3'}$ absorption in the 2'-O-methyl derivatives (37 and 39) causes the $H_{2'}$ and $H_{3'}$ resonances to overlap in the spectra of these compounds. Similar downfield shifts of the $H_{3'}$ signal were also observed in the 2'-deoxy derivatives (38 and 40). The only anomalous shift observed for the absorptions of the 4'- and 5',5"-hydrogens occur with the 3'-deoxy derivative (36) in which the $H_{4'}$ signal is shifted by ~ 0.4 ppm downfield from its usual position. This effect has been noted previously in comparison of the spectra of the 2'- and 3'-deoxy isomers of formycin and formycin B$^{139)}$ and again may be due to the absence of the cis 3'-hydroxyl group and its resulting shielding effect.

The trends, noted above, in the chemical shifts of the sugar hydrogens are interesting and may be of some value in the characterization of other nucleoside derivatives. It is tempting to try to explain the observed shifts in terms of the shielding or deshielding effects of the neighbouring groups, as has been in the cases of the 1'- and 4'-hydrogens,$^{139,140)}$ where a vicinal cis hydroxyl group appears to have a significant shielding effect. The observed trends, however,

are not completely consistent and variations in the conformation of the sugar ring, as evidenced by the variation of the $\underline{H}_{1'}$ coupling constants (see Table IV), may be another important factor in determining the Chemical shifts of the sugar hydrogens.

EXPERIMENTAL

A. GENERAL PROCEDURES

Melting points were determined on a Fischer-Johns apparatus and are uncorrected. Nuclear magnetic resonance (nmr) spectra were recorded on Varian A-60 or HA-100 spectrometers with Me_4Si as internal standard in $Me_2SO\text{-}\underline{d}_6$—$D_2O$ mixed solvent. Ultraviolet (uv) spectra were recorded on Cary 14 or 15 spectrometers with solutions prepared by diluting a 1 ml sample of an accurately determined stock solution in MeOH to 10 ml with MeOH, 0.1 N HCl or 0.1 N NaOH (freshly prepared). Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter using a 10 cm, 1 ml microcell. Mass spectra were determined by the mass spectrometry laboratory of this department on AEI MS-2 or MS-9 instruments at 70 eV. Sample introduction was *via* a direct probe at temperatures between 150 and 230°C. Elemental analyses were obtained by the microanalytical laboratory of this department. Thin layer chromatography (tlc) was performed on Eastman Chromatogram sheets (silica gel No. 13181, indicator No. 6060) or on glass plates coated with Merck silica gel GF-254 with sample observation under uv light (2537 Å). Preparative tlc was performed on glass plates coated with Merck silica gel PF-254. The solvent used for tlc was the upper phase of EtOAc—*n*-PrOH—H_2O (4:1:2) unless

otherwise stated. Evaporations were carried out using a Buchler rotating evaporator with a Dry Ice cooled Dewar condenser under aspirator or oil pump vacuum, at 40°C or less.

All alkylation reactions were carried out in round bottomed flasks protected from moisture by Drierite drying tubes and were stirred magnetically. Filtration by gravity was employed to avoid static charge when hydrocarbon solvents were used.

N,N-Dimethylformamide (DMF) was dried prior to use by distillation from P_2O_5. Skellysolve 'B' was purified by distillation. All other solvents were of reagent grade purity and were used without purification. Linde 4A Molecular Sieves were dried in an oven at 200°C. 9-β-D-Arabinofuranosyladenine (30) was purchased from Pfanstiehl Laboratories. 3-Methyl-2-butenyl bromide was purchased from Chemicals Procurement Laboratories, Inc., and redistilled immediately prior to use. Benzyl bromide was purified by distillation.

B. SYNTHESES

$\underline{N}^6$-(3-Methyl-2-butenyl)adenosine [$\underline{N}^6$-(Δ^2-Isopentenyl)adenosine] (iPA) (22).

(a) With no acid acceptor added: iPA was prepared from adenosine and 3-methyl-2-butenyl bromide according to the procedures described by Grimm and Leonard116) and by Robins et al.69) Although these authors report yields of iPA of 38% and 42.5% respectively, the yields obtained in the present case varied in the range of 20-30%. In agreement with Leonard et al.,60) Martin and Reese,118) and Robins et al.69) it was observed that the $\underline{N}^1$-alkylation step proceeded to about 50-60% and then ceased. However, addition of acid acceptors to the alkylation mixture was found to significantly increase the conversion to the $\underline{N}^1$-alkylated product and resulted in the isolation of higher yields of iPA.

(b) In the presence of 4A molecular sieves: To a solution of 1.96 g (7.5 mmoles) of adenosine in 40 ml of DMF was added 1.79 g (12.0 mmoles) of 3-methyl-2-butenyl bromide and 15 g of Fisher type 4A molecular sieves. The mixture was stirred in the dark for 36 hrs. Tlc showed 80-90% conversion of adenosine to the $\underline{N}^1$-alkylated product. The mixture was diluted

with 15 ml of 3 N NH_4OH and 50 ml H_2O, to bring the pH to ~9 (pHydrion paper), and was then refluxed for 1 hr. Periodic additions of 3 N NH_4OH were made to the refluxing mixture to keep the pH near 9. Tlc showed essentially complete conversion of the N^1- to the N^6-alkylated product. The mixture was filtered and the filter pad washed well with H_2O. The filtrate (~200 ml) was salted with 50 g of NaCl and the resulting solution was extracted with EtOAc (8 x 50 ml). The EtOAc extracts were combined, dried over Na_2SO_4 and the drying agent was filtered off. The filtrate was evaporated and the light brown residual oil was crystallized from MeCN—EtOH (3 crops) to yield 1.41 g (58%) of crude 22. Recrystallization of this material from EtOH—MeCN yielded 0.98 g (41%) of chromatographically pure 22: partial mp. 138-141°, complete at 148-150°.

(c) In the presence of $BaCO_3$: To a solution of 0.40 g (1.5 mmoles) of adenosine in 7 ml of DMF was added 0.32 g (2.2 mmoles) of 3-methyl-2-butenyl bromide and 0.5 g of $BaCO_3$. The mixture was stirred in the dark for 37 hrs. Tlc showed almost complete N^1-alkylation and the mixture was filtered through Celite. The filtrate was evaporated to low volume and diluted with 15 ml of Me_2NH—MeOH (1:1). This solution was stirred for 2 hrs when tlc showed complete conversion of the N^1-alkylated product and a ratio of 80-90% of 22 to 10-20% of

adenosine. The solution was evaporated to low volume, diluted with 20 ml of H_2O and extracted with EtOAc (5 x 20 ml). The combined EtOAc extracts were washed with H_2O (2 x 20 ml) and the combined aqueous washes were extracted with EtOAc (2 x 20 ml). The total combined EtOAc extracts were dried over Na_2SO_4, the drying agent filtered and the filtrate evaporated. The residual oil was dissolved in MeOH and the solution evaporated to yield 0.37 g (74%) of chromatographically pure 22 as a colourless solid. Recrystallization of this material from MeCN—EtOH (3:1) gave 0.29 g (63%) of 22 as colourless needles: mp 135-136°. A small sample of 22 purified by preparative tlc on a silica gel plate and crystallized from the same solvent gave needles of 22: mp 147-149°; $[\alpha]_D^{23}$ -65.4° (c 1, MeOH), -71.5° (c 0.14, EtOH), $[\alpha]_{546}^{23}$ -78.1° (c 1, MeOH), -85° (c 0.14, EtOH); uv max (MeOH) 268 nm (ε 18,100).

Anal. Calcd for $C_{15}H_{21}N_5O_4$: C, 53.72; H, 6.31; N, 20.89. Found: C, 53.79; H, 6.44; N, 20.68.

Numerous samples of 22, prepared by each of the methods outlined above (not including preparative tlc purification), regularly gave melting points in the range 135-144° and these samples consistently gave a low nitrogen analysis. Previously reported melting points were 134-136°,118) 142-143°,60) 145-147°,116) 148°.69) Mass spectral analysis of these samples of

22 showed a peak at m/e 403 which had an intensity in the range 1-4% relative to the parent peak for 22 at m/e 335. This peak is thought to be caused by an impurity in 22 which contains a second 3-methyl-2-butenyl residue. Repeated recrystallization from MeCN—EtOH did not remove this impurity peak and did not consistantly raise the melting point or give a sample of 22 which was suitable for analysis. The analytical sample of 22, which was purified by preparative tlc, did not show any observable peak at m/e 403 in its mass spectrum. Small samples of the impurity material were obtained by column chromatography and the properties of this material are described in the Results and Discussion section.

N^6-(3-Methyl-2-butenyl)-9-β-D-arabinofuranosyladenosine (32).

To a solution of 0.52 g (2.0 mmoles) of 9-β-D-arabinofuranosyladenine (30) in 10 ml of DMF was added 0.45 g (3.0 mmole) of 3-methyl-2-butenyl bromide and 2.5 g of Fisher type 4A molecular sieves. The mixture was stirred in the dark for 19 hr and was then diluted with 10 ml of ~3 N NH_4OH solution and 20 ml of H_2O to bring the pH to ~10.0. The mixture was then refluxed for 1 hr with periodic additions of NH_4OH solution to keep the pH in the range 9.0-10.5. The

mixture was filtered through Celite and the filtrate was diluted with 30 ml of H_2O, salted with 10 g of NaCl and extracted with EtOAc (6 x 25 ml). The extracts were combined and dried over Na_2SO_4. The drying agent was filtered off and the filtrate was evaporated. The resulting syrup was crystallized from i-PrOH to give 0.35 g (54%) of off-white material, mp 158-160°. Recrystallization of this product from i-PrOH—MeOH (7:3) gave colourless flakes of 32, mp 160-161.5°. A sample for analysis was recrystallized from MeCN—EtOH to eliminate i-PrOH of solvation. Pure 32 had mp 161.5-162°; $[\alpha]_D^{28}$ +1.9° (c 1, MeOH); uv max (MeOH) 266.5 nm (ε 18,100).

Anal. Calcd for $C_{15}H_{21}N_5O_4$: C, 53.72; H, 6.31; N, 20.89. Found: C, 53.91; H, 6.01; N, 20.61

N^6-(3-Methyl-2-butenyl)-9-(2-deoxy-β-D-erythro-pentofuranosyl)adenine (2'-Deoxy-N^6-(Δ^2-isopentenyl)-adenosine) (34).

To a solution of 5.02 g (20.0 mmoles) of 2'-deoxyadenosine (10) in 100 ml of DMF was added 4.47 g (30.0 mmole) of 3-methyl-2-butenyl bromide and 25 g of Fisher type 4A molecular sieves. The mixture was stirred in the dark for 46 hrs and was then filtered through Celite. The filter pad was washed well with DMF and the filtrate was evaporated to ~50 ml. The

resulting golden/yellow solution was diluted with 200 ml of Me_2NH—MeOH (1:1) and stirred magnetically. After 4 hrs the solution was evaporated to low volume, diluted with 200 ml of saturated NaCl solution and extracted with $CHCl_3$ (3 x 100 ml). The combined $CHCl_3$ extracts were washed with H_2O (3 x 100 ml) and dried over Na_2SO_4. The drying agent was filtered off and the filtrate was evaporated to yield a yellow syrup of crude 34. The syrup was dissolved in a minimal volume of $CHCl_3$ and applied to a dry packed column (2.3 x 74 cm, 300 g) of Woelm alumina (deactivated to grade III). The column was eluted with $CHCl_3$—MeOH (50:1). Fractions containing 34 were combined, evaporated and dissolved in 200 ml of MeOH—H_2O (1:3). This solution was extracted with Skellysolve B (1 x 100 ml) and with EtOAc (5 x 100 ml). The Skellysolve B extract contained only coloured impurities and was discarded. The latter EtOAc contained pure 34 and were set aside. The first EtOAc extract contained 34 contaminated with minor impurities and was evaporated. The residue was subjected to the above extraction procedure (omitting the Skellysolve B extraction) and one further repetition of this procedure yielded EtOAc extracts containing only 34. The total EtOAc extracts were then combined, evaporated and coevapo-

rated with EtOH and then benzene to yield a syrup of pure 34. This syrup was dissolved in a minimal quantity of benzene containing EtOH to affect solution and was freeze-dried at -78°. After 15 hr 1.75 g of crystalline solid remained in the flask. This product was recrystallized from acetone-cyclohexane (3 crops) to give 1.33 g (23%) of colourless needles of 34: mp 106-109°; $[\alpha]_D^{28}$ -19.5° (*c* 1, MeOH); uv max (MeOH) 268 nm (ε 18,900). (Reported:$^{95)}$ mp 48-49°; $[\alpha]_D^{25}$ -9.9° (*c* 0.8, EtOH)).

Anal. Calcd for $C_{15}H_{21}N_5O_3$: C, 56.41; H, 6.63; N, 21.93. Found: C, 56.27; H, 6.93; N, 21.59.

N^6-Benzyladenosine (29).

To a cooled solution, prepared by warming and cooling, of 1.0 g (3.8 mmoles) of adenosine in 20 ml of DMF was added 1.92 g (11.0 mmoles) of benzyl bromide and the solution was stirred at 40° for 48 hr. The solution was then evaporated to ~1 ml and this was added dropwise to 100 ml of dry acetone with vigorous stirring. Dry Et_2O (200 ml) was added and the precipitate was collected by centrifugation. This material was dissolved in 25 ml of MeOH and 25 ml of Me_2NH—MeOH (1:1) was added. This solution was stirred for 4 hr, evaporated and coevaporated with additional MeOH. The residue was dissolved in 25 ml

of warm MeOH, H_2O (150 ml) was added and the resulting solution was extracted with EtOAc (7 x 50 ml). The combined EtOAc extracts were dried over Na_2SO_4 and the drying agent was filtered off. The filtrate was evaporated and the residue was coevaporated twice with EtOH and then an additional time to a volume where crystallization began. This mixture was allowed to stand at 4° for 18 hr to give 0.88 g (67%) of colourless granules of 29, mp 167.5-168°. A sample for analysis was recrystallized from EtOH to give crystals, mp 186-187° with partial melting at 169-170°, followed by resolidification: $[\alpha]_D^{28}$ -65.1° (*c* 1, MeOH); uv max (MeOH) 270 nm (ε 20,800), 266.5 nm (sh, ε 20,500). (Reported:[96] mp 183°; $[\alpha]_D^{25}$ -61.7° (*c* 0.227, 95% EtOH)).

Anal. Calcd for $C_{17}H_{19}N_5O_4$: C, 57.13; H, 5.36; N, 19.60. Found: C, 56.98; H, 5.40; N, 19.85.

N^6-Benzyl-9-β-D-arabinofuranosyladenine (33).

To a solution of 1.87 g (7.0 mmoles) of 9-β-D-arabinofuranosyladenine (30) in 30 ml of DMF was added 3.13 g (21.0 mmoles) of benzyl bromide. The resulting solution was stirred for 68 hr and was then evaporated to a volume ~5 ml. This solution was added dropwise to 100 ml of vigorously stirred dry acetone, and anhydrous Et_2O (200 ml) was added to

complete the precipitation. The precipitate was allowed to settle overnight and the solvent was decanted off. The precipitate was dissolved in 20 ml of MeOH and 30 ml of Me_2NH:MeOH (1:1) was added. This solution was stirred 3 hr, evaporated to low volume and diluted with EtOAc (25 ml) and H_2O (50 ml). Crystallization occurred in the EtOAc layer and the organic phase containing the crystalline material was separated. The aqueous phase was extracted with EtOAc (6 x 25 ml) and all of the organic extracts (including that one containing crystalline material) were combined and evaporated. The residue was coevaporated twice with EtOH and the resulting pale yellow solid was crystallized from EtOH to give 1.72 g (69%) of colourless needles of 33, mp 198.5-199.5°. Recrystallization from EtOH yielded an analytical sample which had the same melting point: $[\alpha]_D^{28}$ + 2.8° (*c* 1, MeOH); uv max (MeOH) 267 nm (ε 20,000).

Anal. Calcd for $C_{17}H_{19}N_5O_4$: C, 57.13; H, 5.36; N, 19.60. Found: C, 57.39; H, 5.49; N, 19.51.

N^6-Benzyl-9-(2-deoxy-β-*D*-*erythro*-pentofurnaosyl)adenine (*N*^{6}-Benzyl-2'-deoxyadenosine) (35).

To a solution of 2.51 g (10.0 mmoles) of anhydrous 2'-deoxyadenosine (10) in 50 ml of DMF was

added 3.60 g (20.0 mmoles) of benzyl bromide. After stirring for 5 days the resulting solution was evaporated to low volume and then diluted with 50 ml of Me_2NH—MeOH (1:1). This solution was stirred for 1 hr and then evaporated to low volume and diluted with 100 ml of H_2O. The resulting emulsion was extracted with $CHCl_3$ (5 x 50 ml) and the extracts were combined and dried over Na_2SO_4. The drying agent was filtered off and the filtrate was evaporated. The resulting syrup was dissolved in $CHCl_3$ and the solution was made up to 100 ml with $CHCl_3$. A 70 ml portion of this solution was evaporated and the residue dissolved in a minimal volume of $CHCl_3$—MeOH (39:1). This solution was applied to a column (1.7 x 60 cm, 150 g) of Woelm alumina (deactivated to grade III) packed in $CHCl_3$:MeOH (39:1). The column was eluted with the same solvent and the product containing fractions were combined and evaporated to give 1.29 g (58%) of 35 as a colourless solid foam. Crystallization of this material from EtOH yielded 0.94 g (42%, based on 1.76 g of starting 10): mp 175.5-176.5°; $[\alpha]_D^{28}$ -19.3° (c 1, MeOH); uv max (MeOH) 270 nm (ε 21,800), 267 nm (sh, ε 21,600).

Anal. Calcd for $C_{17}H_{19}N_5O_3$: C, 59.81; H, 5.61; N, 20.52. Found: C, 60.15; H, 5.84; N, 20.80.

N^6-Benzyl-9-(3-deoxy-β-D-erythro-pentofuranosyl)adenine (N^6-Benzyl-3'-deoxyadenosine) (36).

To a solution of 0.905 g (3.6 mmoles) of 3'-deoxyadenosine (31)$^{123)}$ in 25 ml of DMF was added 12.5 g (7.2 mmoles) of benzyl bromide. After stirring for 6 days the resulting solution was evaporated to low volume and diluted with 35 ml of Me_2NH—MeOH (1:1). This solution was stirred for 3 hr and then evaporated to low volume, diluted with 200 ml of EtOAc and 100 ml of H_2O was added. The organic phase was separated and the aqueous phase was extracted with a further 100 ml of EtOAc. The combined organic phase was washed with 75 ml of H_2O, dried over Na_2SO_4 and filtered. The filtrate was evaporated to the point of crystallization and then allowed to stand at 4° for 14 hr. Filtration gave 0.75 g (65%) of colourless granules of 36; mp 185-186°. An analytical sample was recrystallized from EtOH: mp 185-187°; $[\alpha]_D^{28}$ -49.1° (c 1, MeOH); uv max (MeOH) 271 nm (ε 21,000), 267 nm (sh, ε 20,900).

Anal. Calcd for $C_{17}H_{19}N_5O_3$: C, 59.81; H, 5.61; N, 20.52. Found: C, 59.97; H, 5.83; N, 20.27.

N^6-(3-Methyl-2-butenyl)-2'-O-methyladenosine (37) and N^6-(3-Methyl-2-butenyl)-3'-O-methyladenosine (38).

To a stirred suspension of 4.50 g (13.3 mmoles)

of $\underline{N}^6$-(3-methyl-2-butenyl)adenosine (22) in 150 ml of a 10^{-3} M solution of $SnCl_2 \cdot 2H_2O$ in MeOH[127)] was slowly added a stock solution[141)] of diazomethane in 1,2-dimethoxyethane (glyme) until the solution became clear and a faint yellow colour persisted. Tlc ($CHCl_3$—MeOH, 95:5) showed monomethylation to be complete. The solution was evaporated to leave a pale yellow solid foam which was dissolved in 30 ml of glyme—H_2O (1:1) by warming on the steam bath. Upon cooling this solution, 2.76 g (59%) of the 3'-isomer (38) containing traces of the 2'-isomer (37) crystallized (mp 109-111°) and was filtered. This material was boiled with acetone and filtered hot through Celite. The filter pad was washed well with boiling acetone and the combined filtrate was concentrated to about 20 ml and allowed to cool at 4°. The product which crystallized was recrystallized from 10 ml of acetone to give 1.82 g (39%) of colourless granules of 38, mp 112.5-113.5° (powdered and dried at 78° (0.1 mm) for 24 hr over P_2O_5 and Paraffin wax).

The mother liquor from crystallization was evaporated and the resulting solid foam was dissolved in 5 ml of EtOH—H_2O (30:70). This solution was applied to column (3.3 x 120 cm, 1000 ml) of Dowex 1-X2(OH^-) (200-400 mesh) packed in EtOH—H_2O (30:70).

The column was eluted with the same solvent and 20 ml fractions were collected at the rate of 1 ml/min. Fractions 110-189 were combined, evaporated and coevaporated with EtOH and $CHCl_3$ to give 1.44 g (31%) of 37 as a solid foam. This material was dissolved in 75 ml of MeOH—H_2O (1:4) and the resulting solution was extracted with Skellysolve B (2 x 40 ml) and EtOAc (4 x 40 ml). The Skellysolve B extracts were discarded and the EtOAc extracts were combined and washed with H_2O (2 x 50 ml). The organic phase now contained no visible slower migrating spots on tlc and was set aside. The combined aqueous washes were extracted with EtOAc (2 x 50 ml) and the combined organic phase was back washed with H_2O (50 ml) and then added to the above EtOAc extracts. This combined solution was evaporated and the residue was dissolved in 25 ml of MeOH—H_2O (1:4) and extracted with Et_2O (10 x 25 ml). The first Et_2O extract was contaminated with a trace of faster migrating material on tlc. The additional Et_2O extracts were combined, dried over Na_2SO_4, filtered, evaporated and coevaporated with benzene. A benzene solution of the oily residue was freeze-dried at -78° to give 0.48 g of chromatographically pure 37: mp 47-60°, complete at 85°; $[\alpha]_D^{28}$ -68.9° (c 1, $CHCl_2$); uv max (MeOH) 268 nm (ε 18,400).

Anal. Calcd for $C_{16}H_{23}N_5O_4$: C, 55.00; H, 6.63; N, 20.05. Found: C, 54.98; H, 6.84; N, 19.86.

Fractions 445-595 were combined, evaporated and coevaporated with EtOH and then $CHCl_3$ to give 0.28 g of 38 as a solid foam. This material was dissolved in boiling acetone and filtered hot through Celite. The filter pad was washed with EtOH and the combined filtrate was evaporated to a solid residue which was crystallized from acetone to give 0.13 g (3%) of 38 (total yield of pure crystalline 38, 1.95 g (42%)): mp 113-114°; $[\alpha]_D^{28}$ -76.0° (c 1, MeOH); uv max (MeOH) 268 nm (ε 19,700).

Anal. Calcd for $C_{16}H_{23}N_5O_4$: C, 55.00; H, 6.63; N, 20.05. Found: C, 54.71; H, 6.78; N, 20.00.

N^6-Benzyl-2'-O-methyladenosine (39) and N^6-Benzyl-3'-O-methyladenosine (40).

A stirred suspension of N^6-Benzyladenosine (29) in 300 ml of a 10^{-3} M solution of $SnCl_2 \cdot 2H_2O$ in MeOH was converted to a mixture of 39 and 40 as described above for the preparation of 37 and 38. The syrup which resulted after evaporation was dissolved in 10 ml of EtOH—H_2O (3:7) and applied to a column (3.3 x 130 cm, ~1100 ml) of Dowex 1-X2(OH^-) (200-400 mesh) packed in the same solvent. The column was

eluted initially with EtOH—H_2O (3:7, ~11 ℓ) and 20 ml fractions were collected at a rate of 1 ml/min. Fractions containing pure 39 were combined, evaporated and coevaporated with EtOH and then $CHCl_3$ to yield 2.76 g (36%) of 39 as a pale yellow solid foam. This material (7.4 mmole) was dissolved in 6.5 ml of EtOH and 16 ml of acetone and was treated with a solution of 0.27 g (7.4 mmoles) of HCl gas in 3.2 ml of EtOH and 16 ml of acetone. An additional 65 ml of acetone was added and the mixture was allowed to stand at 4° for 15 hr to yield 2.37 g (28%) of crystalline 39 hydrochloride, mp 129-132°. A sample for anlysis was recrystallized from dry EtOH—Et_2O containing 0.37 equiv. of HCl to give crystals with mp 147.5-149.5°: $[\alpha]_D^{28}$ -44.1° (*c* 1, MeOH); uv max (MeOH) 271 nm (ε 20,100), 267 nm (sh, ε 20,000), (NaOH) 269.5 nm (ε 20,000).

Anal. Calcd for $C_{18}H_{21}N_5O_4 \cdot HCl$: C, 53.00; H, 5.44; N, 17.17; Cl, 8.69. Found: C, 52.89; H, 5.73; N, 16.89; Cl, 8.70.

Elution of the column was continued and the concentration of EtOH in the eluant was gradually increased (over a volume ~5 ℓ) to EtOH—H_2O (8:2). The 74 fractions following the elution of pure 39 were combined and evaporated to give 0.45 g (6%) of a mixture of 39 and 40 as a solid foam. The following

fractions containing pure 40 were combined, evaporated and coevaporated with EtOH and $CHCl_3$ to give 4.50 g (58%) of a solid foam which was crystallized from acetone to yield 3.68 g (47%) of colourless granules of 40: mp 141-142.5° (dried for 15 hr at 60° (0.1 mm) over P_2O_5 and Paraffin wax); $[\alpha]_D^{28}$ -44.1° (*c* 1, MeOH); uv max (MeOH) 271.5 nm (ε 20,300).

Anal. Calcd for $C_{18}H_{21}N_5O_4$: C, 58.21; H, 5.70; N, 18.86. Found: C, 58.14; H, 5.63; N, 19.16.

3-Methyl-2-butenylamine.

This was prepared from 3-methyl-2-butenyl bromide in a similar manner to that used by Leonard *et al*[142)] for the conversion of the *p*-toluenesulphonate derivative of 3-methyl-3-butenyl alcohol to 3-methyl-3-butenylamine:

N-(3-Methyl-2-butenyl)phthalimide.

To a suspension of 18.5 g (100.0 mmole) of potassium phthalimide in 100 ml of DMF was added 12.7 g (85.0 mmole) of 3-methyl-2-butenyl bromide and the mixture was stirred for 20 hr at room temperature. The resulting clear solution was poured into 500 ml of ice cold H_2O and the crystalline solid produced was filtered and dried on the filter. Recrystallization of this material from Skellysolve B yielded (2 crops) 16.3 g (89%) of product as a colourless

crystalline solid: mp 100-102°; nmr ($CDCl_3$) δ 7.72 (m, 4, aromatic H), 5.25 (t, J ~ 7 Hz, 1, =CH-), 4.24 (d, J ~ 7 Hz, 2, $-CH_2-$), 1.70, 1.82 (s, s, 6, $C(CH_3)_2$).

3-Methyl-2-butenylamine.

To a suspension of 14.0 g (65.0 mmole) of N-(3-methyl-2-butenyl)phthalimide in 250 ml MeOH was added 4.2 ml (70 mmole) of an 85% solution of hydrazine hydrate. The mixture was heated under reflux for 3 hr and the resulting clear solution was evaporated. The residue was dissolved in 300 ml of ice cold H_2O and acidified to pH ~ 1.5 using concentrated HCl. The resulting thick white precipitate was filtered through Celite and the filtrate was evaporated and coevaporated with EtOH. The residue was dissolved in EtOH, filtered and the filtrate was made up to 100 ml with EtOH. This solution was cooled in an ice bath and 900 ml of Et_2O was gradually added with stirring. Pale brown crystalline flakes were filtered off and a second crop was obtained by dilution of the filtrate with a further 1000 ml of Et_2O to give a total of 6.51 g (81%) of 3-methyl-2-butenylamine hydrochloride. This material was recrystallized from EtOH—Et_2O, using the same procedure as above, to yield 5.99 g (76%), mp 188-196° (dec.). A 5.0 g (41.1 mmole) portion of this material was dissolved in 20 ml of H_2O and cooled in an ice bath. To this solution was added a solution of 1.65 g

of NaOH in 20 ml of H_2O and the resulting solution was extracted with Et_2O (2 x 40 ml). The combined organic phase was dried over Na_2SO_4 and filtered. The filtrate was evaporated to remove solvent and the residual liquid was distilled at reduced pressure to give 3-methyl-2-butenylamine: bp 32-34°/36 mm; n_D^{25} 1.4422. (Reported:[143] n_D^{25} 1.4420).

7-N-(3-Methyl-2-butenyl)amino-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine (N^6-(Δ^2-Isopentenyl)-formycin) (42).

A solution of 0.80 g (2.8 mmole) of 7-Chloro-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine (41)[144] in 2.7 g (32 mmole) of 3-methyl-2-butenylamine was stirred for 2.5 hr at room temperature while protected from moisture. The resulting solution was poured into 25 ml of ice cold 1 N NaOH and this solution was extracted with Et_2O (5 x 20 ml) to remove excess amine. The organic phase was discarded and ice was added to the aqueous phase which was then cautiously neutralized to pH 6 (pHydrion paper), with concentrated HCl, and extracted with EtOAc (15 x 20 ml). The combined organic phase was dried over Na_2SO_4, filtered and evaporated. The colourless solid residue was dissolved in MeOH and filtered through Celite.

The filtrate was evaporated to give 0.73 g (77%) of 42 as a solid foam which was treated with 20 ml of acetone and allowed to stand overnight. Upon agitation the product crystallized and two crops of 42, 0.51 g (55%), mp 192-193° and 0.09 g (9%), mp 165-173°, were obtained. A sample for analysis was recrystallized from acetone—EtOH (2:1) to give colourless crystals of 42: mp 193.5-195° (lit.[102] mp 120-122°); $[\alpha]_D^{28}$ -55.6° (c 1, MeOH); uv max (MeOH) 296, 238.5 nm (ε 16,000, 6,300), shoulders 307, 288 nm (ε 11,700, 14,000), (NaOH) 300, 243.5 nm (ε 12,200, 13,200), (HCl) 311, 301, 239 nm (ε 16,500, 17,300, 6,600).

Anal. Calcd for $C_{15}H_{21}N_5O_4$: C, 53.72; H, 6.31; N, 20.89. Found: C, 53.44; H, 6.25; N, 21.09.

7-N-Benzylamino-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine (N^7-Benzylformycin) (43).

A solution of 0.50 g (1.8 mmole) of (41)[144] in 5 ml of benzylamine was stirred for 2.5 hr at room temperature while protected from moisture. The resulting solution was poured into 25 ml of ice cold 1 N NaOH and this solution was extracted with Et_2O (5 x 25 ml) to remove excess amine. The organic phase was discarded and the aqueous phase was acidified

to pH 5 (pHydrion paper) with 1 N HCl solution and extracted with EtOAc (10 x 50 ml). The combined organic phase was dried over Na_2SO_4, filtered and evaporated. Coevaporation of the residue with EtOH gave 0.52 g (80%) of crude 43 as a yellow solid foam. This material was dissolved in a minimal volume of MeOH and purified by preparative thin layer chromatography on three silica gel plates (18 x 18 cm). After development the appropriate bands were removed from the plates and the silica gel was washed with MeOH (4 x 60 ml) to extract the product. The extracts were combined, filtered through Celite and evaporated. The residue was dissolved in $CHCl_3$ containing a little EtOH, filtered through Celite and the clear filtrate was evaporated to give 0.44 g (70%) of 43 as a colourless solid foam. This material was dissolved in 1 ml of 2 N HCl in EtOH and 0.34 g (50%) of 43·HCl crystallizaed as colourless granules, mp 206-209° (some decomposition). A sample for analysis was recrystallized from EtOH—MeOH (containing 2.0 equiv. of HCl) to give fine needles of 43·HCl: mp 206-212° (some decomposition); $[\alpha]_D^{28}$ -28.8° (c 0.5, MeOH); uv max (MeOH) 307, 297, 289 (sh) and 239 nm (ε 11,900, 14,800, 13,000 and 6,800), (HCl) 301 and 291 nm (ε 15,500 and 16,100), (NaOH) 305 and 243 nm (ε 10,700 and 12,500).

Anal. Calcd for $C_{17}H_{19}N_5O_4 \cdot HCl$: C, 51.84, H, 5.12; N, 17.79; Cl, 9.00. Found: C, 51.92; H, 5.31; N, 17.49; Cl, 9.21.

6-(3-Methyl-2-butenyl)thio-9-β-D-ribofuranosylpurine (47)

To a solution of 1.50 g (5.3 mmole) of 6-thiopurine riboside (45) in 15 ml of DMF was added 1 g of anhydrous K_2CO_3 and 0.82 g (5.5 mmole) of 3-methyl-2-butenyl bromide. The mixture was stirred for 1 hr at room temperature and was then partitioned between H_2O (150 ml) and EtOAc (3 x 50 ml). The combined organic phase was dried over Na_2SO_4, filtered and the filtrate evaporated. The residue was partitioned between H_2O (100 ml) and $CHCl_3$ (2 x 50 ml) and the combined organic phase was dried over $MgSO_4$, filtered through Celite and the filtrate evaporated to a volume of 30 ml. This solution was added dropwise to 400 ml of vigorously stirred Skellysolve B. The resulting precipitate was filtered by gravity and dried at 0.1 mm and room temperature over P_2O_5 and Paraffin wax to give 1.56 g (82%) of colourless solid 47: mp 72-76°; $[\alpha]_D^{28}$ -52.7° (*c* 1, MeOH); uv max (MeOH) 292 nm (ε 20,900), 287 nm (sh, ε 20,400), (HCl) 296 nm (ε 18,300), (NaOH) 295 nm (ε 18,700).

Anal. Calcd for $C_{15}H_{20}N_4O_4S$: C, 51.20; H, 5.71; N, 15.90; S, 9.09. Found: C, 50.91; H, 5.66; N, 16.05; S, 8.83.

2-Amino-6-(3-methyl-2-butenyl)thio-9-β-D-ribofuranosylpurine (S^6-(3-Methyl-2-butenyl)-6-thioguanosine) (48).

To a solution of 1.50 g (5.0 mmole) of 6-thioguanosine (46) in 15 ml of DMF was added 1.5 g of anhydrous K_2CO_3 and 0.76 g (5.1 mmole) of 3-methyl-2-butenyl bromide. The resulting mixture was stirred for 1 hr at room temperature and was then partitioned between H_2O (150 ml) and EtOAc (6 x 50 ml). The combined organic phase was dried over Na_2SO_4, filtered and evaporated. The residue was partitioned between H_2O (100 ml) and $CHCl_3$ (4 x 50 ml). Gel formation occurred in the first extract and this was dissolved by warming and diluting with EtOAc. The combined organic phase was then dried over $MgSO_4$, filtered through Celite, evaporated and coevaporated with $CHCl_3$. The residual syrup was dissolved in 30 ml of $CHCl_3$ and added dropwise to 400 ml of vigorously stirred Skellysolve B. The resulting precipitate was filtered by gravity and dried at room temperature and 0.1 mm over P_2O_5 and Paraffin wax to

give 1.85 g (85%) of 48 as a powder. Dissolution of this material in 30 ml of $CHCl_3$ and reprecipitation into 400 ml of Skellysolve B yielded 1.65 g (76%) of 48: mp 87-92°; $[\alpha]_D^{28}$ -45.1° (*c* 1, MeOH); uv max (MeOH) 248 nm (ε 14,600), 303 nm (ε 13,300), (HCl) 250 nm (ε 9,200), 327 nm (ε 11,700), (NaOH) 248 nm (ε 12,200), 313.5 (ε 13,300).

Anal. Calcd for $C_{15}H_{21}N_5O_4S$: C, 49.05; H, 5.76; N, 19.09; S, 8.74. Found: C, 49.27; H, 5.52; N, 19.04; S, 8.49.

CHAPTER II

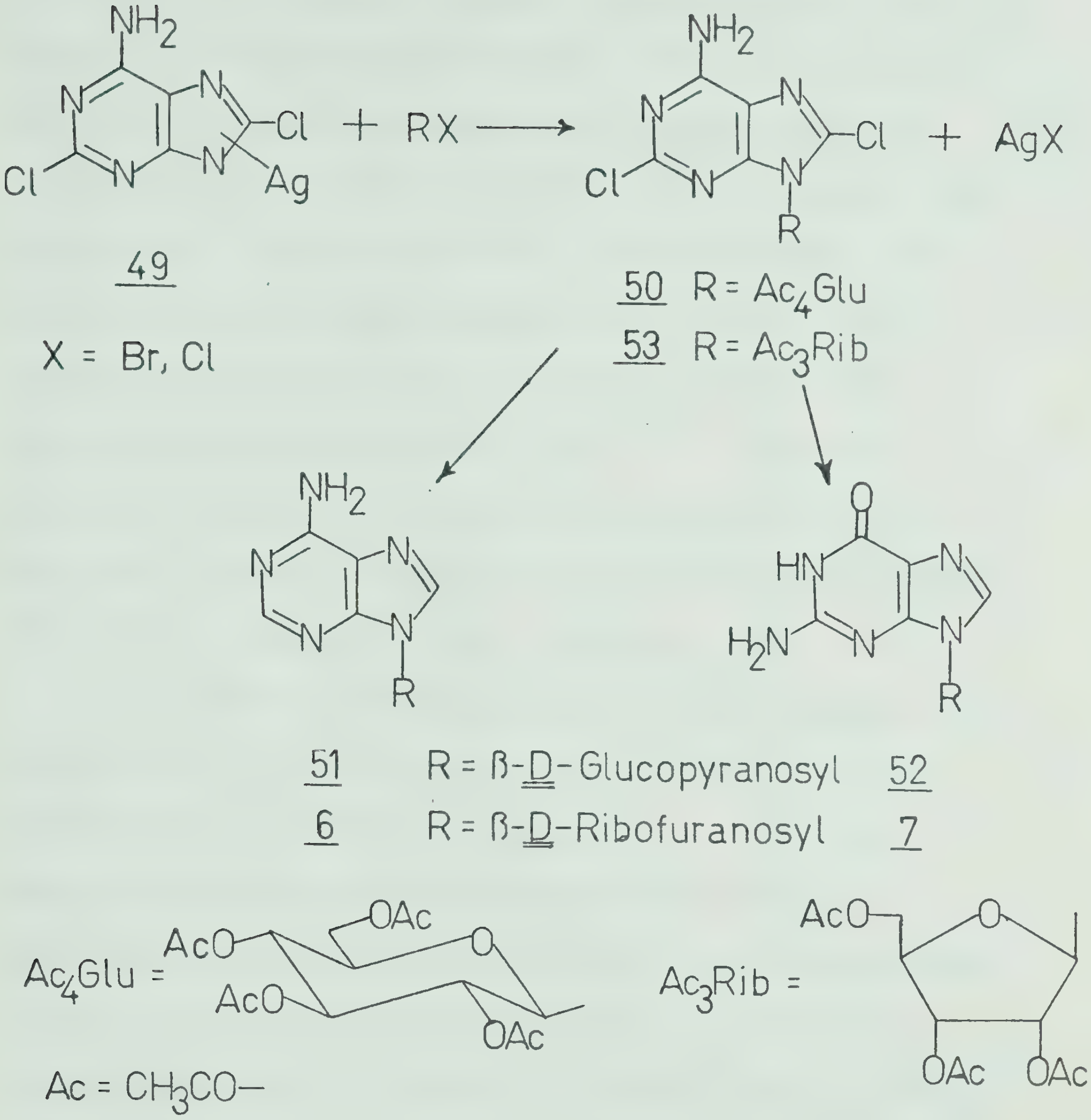
SYNTHESIS OF 2'-DEOXYNUCLEOSIDES AND 2-DEOXYRIBOFURANOSIDES

INTRODUCTION

The synthesis of nucleosides by base-sugar coupling procedures was pioneered by Fischer and Helferich[145] in 1914. Reaction of the silver salt of theophylline with tetra-O-acetyl-α-D-glucopyranosyl bromide yielded a glycosylpurine derivative which was later shown to be 7-substituted. Similar reaction with the silver salt of 2,8-dichloroadenine (49) gave, after deacetylation, 2,8-dichloro-9-D-glucopyranosyladenine (50). Complete dehalogenation of 50 yielded 9-β-D-glucopyranosyladenine (51), partial dehalogenation followed by nitrous acid deamination and treatment with alcoholic ammonia gave 9-β-D-glucopyranosylguanine (52) (see Scheme VIII).

Synthesis of the naturally occurring purine ribonucleosides was not achieved until Todd and coworkers[19] developed the synthesis of a suitable ribofuranosyl halide in 1948. Thus condensation of tri-O-acetyl-D-ribofuranosyl chloride with 49 gave the 2,8-dichloroadenosine derivative, 53. Conversion of 53 to adenosine (6) and guanosine (7) was carried out

SCHEME VIII



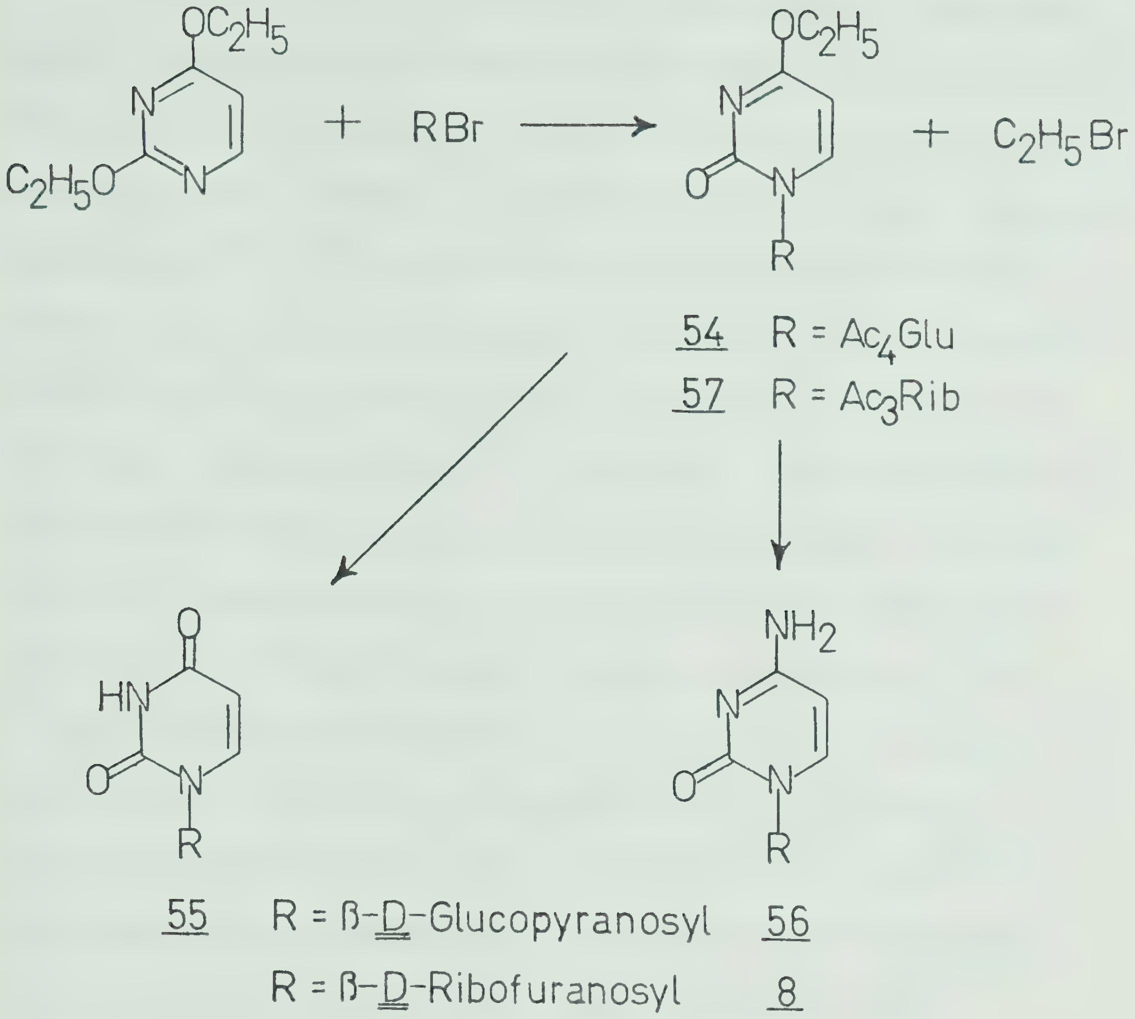
in analogous fashion to that used by Fischer and Helferich.

Attempts by Fischer and Helferich to apply their procedure to pyrimidines failed. Work by Levene and Sobotka[146)] indicated that use of the silver salts of

pyrimidines resulted in O- rather than N-glycosylation. Hilbert and Johnson overcame this problem by the use of 2,4-dialkoxypyrimidines. Thus, condensation of 2,4-diethoxypyrimidine with tetra-O-acetyl-D-glucopyranosyl bromide afforded the nucleoside intermediate 54. Treatment of 54 with methanolic HCl yielded 1-β-D-glucopyranosyluracil (55)[147] and ammonolysis of 54 using alcoholic ammonia gave 1-β-D-glucopyranosyl cytidine (56).[148] Analogous reactions using tri-O-acetyl-D-ribofuranosyl bromide gave the naturally occurring ribonucleoside cytidine (8), in low yield[149] (see Scheme IX).

A number of modifications of these basic procedures have been reported with improved results. Davoll and Lowy[150] demonstrated that monochloromercury derivatives of certain purines could be employed to greater advantage than the corresponding silver salts in the Fischer-Helferich procedure. Also, acylation of the basic 6-amino group of adenosine allows the use of this derivative in condensation reactions. Thus, reaction of the chloromercuri derivative of 6-benzamidopurine with tri-O-acetyl-D-ribofuranosyl chloride, followed by deacylation, gave adenosine. Fox and coworkers[151] employed dithyminylmercury in a synthesis of 9-β-D-ribofuranosylthymine. They also noted that use of tri-O-benzoyl-D-ribo-

SCHEME IX



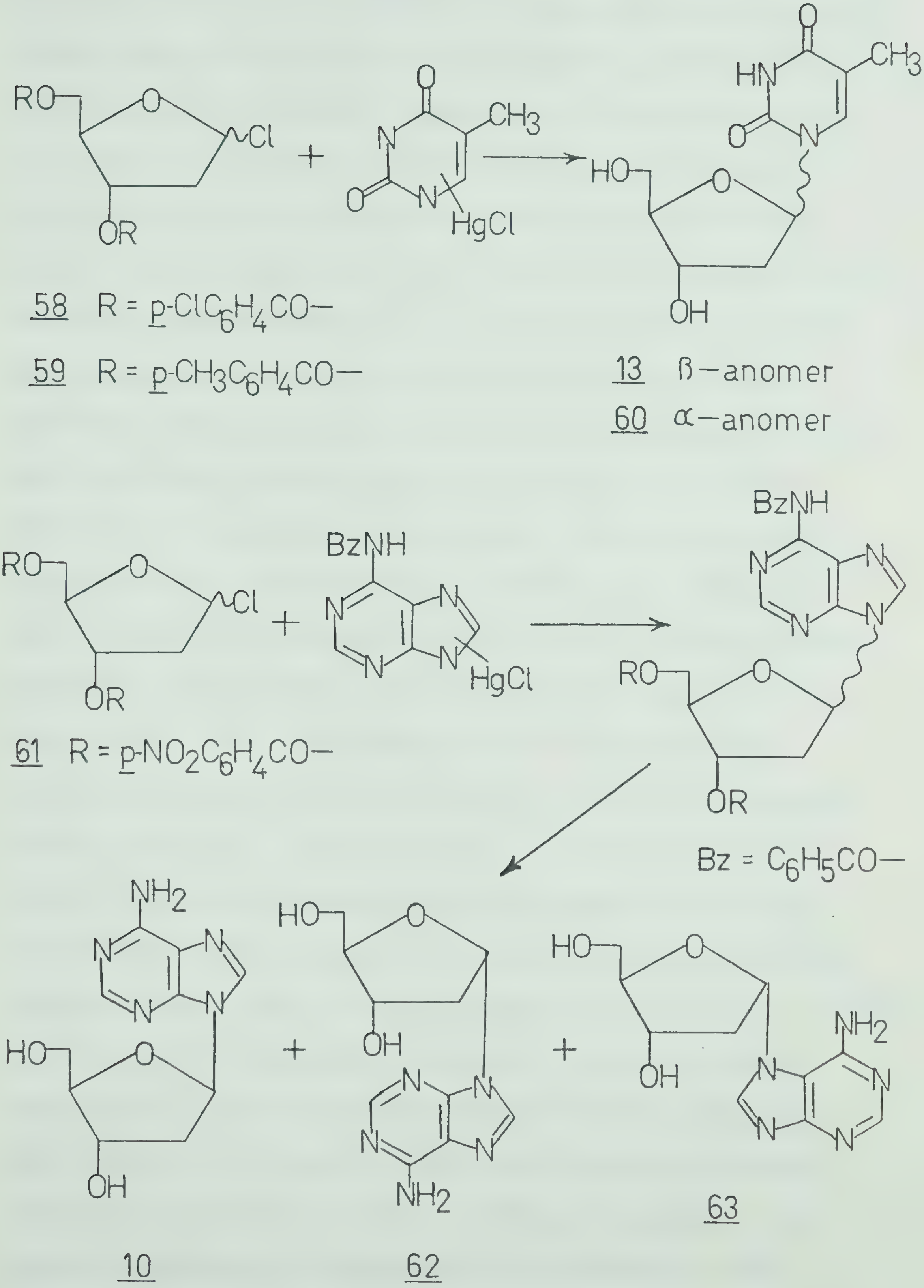
furanosyl chloride as the sugar component in the condensation reaction gave better yields than the corresponding bromo or tri-O-acetyl derivatives. Improved preparations of tri-O-acyl-ribofuranosyl halides and subsequent modifications of the basic procedure have made the sugar-base coupling approach the most convenient and efficient synthetic route to naturally occurring ribonucleosides as well as a host

of analogues.[21,22,152-156]

Initial attempts to adapt this general approach to the synthesis of 2'-deoxynucleosides were unsuccessful due to the extreme lability of the poly-O-acyl-2-deoxyglycosyl halides. Almost simultaneously, two groups of workers[157,158] discovered that by using blocking groups not normally employed, stable di-O-acyl-2-deoxy-D-ribofuranosyl chlorides (58, 59 and 61) could be obtained. Hoffer et al[157] prepared 2'-deoxy-α and β-D-ribofuranosylthymine (60 and 13 respectively) in good yield by condensation of the chloromercuri derivative of thymine with 58 or 59. Anomeric mixtures of the 2'-deoxynucleoside derivatives of cytosine, 5-fluorouracil and 5-fluorocytosine were also obtained in an analogous manner.[157,159] Ness and Fletcher[158] condensed the chloromercuri derivative of 6-benzamidopurine with 61 to give the α and β anomers of 2'-deoxyadenosine (62 and 10). Also obtained was a small amount of a third 2'-deoxynucleoside derivative which was tentatively assigned the structure 2'-deoxy-7-α-D-ribofuranosyladenine (63) (see Scheme X). Following this initial work, many reports of the syntheses of 2'-deoxynucleosides and numerous analogues, by various base-sugar coupling procedures, have appeared in the literature.[160-174]

In contrast to the preparations of ribonucleo-

SCHEME X



58 $R = \underline{p}\text{-}ClC_6H_4CO\text{—}$

59 $R = \underline{p}\text{-}CH_3C_6H_4CO\text{—}$

13 β—anomer

60 α—anomer

61 $R = \underline{p}\text{-}NO_2C_6H_4CO\text{—}$

$Bz = C_6H_5CO\text{—}$

10

62

63

sides, which generally give rise to a single anomeric product, 2'-deoxynucleoside syntheses almost invariably yield mixtures of both α and β anomers. Baker and coworkers[175)] noted that condensation reactions of glycosyl halides having a 2-acyl substituent with metal salts of purine or pyrimidine bases gave nucleosidic products having only the C-1'—C-2' trans configuration. This has been explained[175,176)] in terms of neighbouring group participation by the 2-acyl group of the glycosyl halide. In the absence of the 2-acyl substituent no steric control of the reaction is in effect and anomeric mixtures are produced. This situation has been used to advantage by Khorana and coworkers[177)] who utilized 5-O-benzoyl-2,3-O-carbonyl-D-ribofuranosyl bromide, a sugar derivative not having a participating group at C2, in a synthesis of the α-D-isomer of adenosine (which was separated from the simultaneously formed β-anomer). Kotick et al,[178)] studied modified Hilbert-Johnson condensations of a number of bis-trimethylsilyluracil derivatives using the pure α-anomer of 58 and were able to control the anomeric ratio in the products to an appreciable extent. Reaction, under conditions in which the trimethylsilyl chloride produced during the reaction was azeotropically removed, yielded the β-anomer as the predominant product. Conversly, addition of trimethylsilyl chloride to the

reaction mixture gave predominant α-anomer formation.

Modification of preformed ribonucleosides is another technique that has been used in the synthesis of deoxynucleosides. However the 2'-deoxy isomers are usually the most difficult to obtain and techniques that have been developed for their synthesis are not generally applicable. The first reported synthesis of a 2'-deoxynucleoside was by Brown et al.[179)] They found that treatment of 5'-O-acetyl-2'-O-p-toluenesulphonyluridine with sodium iodide yielded the 2'-iodo derivative. Subsequent hydrogenation and deacetylation gave 2'-deoxyuridine and thymidine was also prepared in an analogous manner from 9-β-D-ribofuranosylthymine. These workers showed[180)] that the halogenation reaction went via an O^2,2'-anhydropyrimidine derivative and Fox and coworkers[181)] subsequently reported high yield syntheses of 2'-halopyrimidine nucleosides from O^2,2'-anhydropyrimidines. Holý[182,183,184)] has employed O^2,2'-anhydropyrimidine nucleosides, prepared by the elegant procedure developed by Sanchez and Orgel,[185)] in syntheses of numerous 2'-halo and 2'-deoxypyrimidine nucleosides. Recently Ponpipom and Hanessian[186)] have reported a facil synthesis of 2',5-dibromouridine by treatment of 2',3'-O-benzylideneuridine with N-bromosuccinimide. This reaction again proceeds via the O^2,2'-anhydro derivative

and the product is readily converted into 2'-deoxyuridine.

Entry into 2'-halo and 2'-deoxy purine nucleoside derivatives is more difficult. Ikehara and Tada[187)] have reported the synthesis of 8,2'- and 8,3'-anhydro-8-mercaptoadenosine derivatives. Raney nickel desulphurization of these compounds yielded 2'- and 3'-deoxyadenosine, respectively. Both Robins and coworkers[123,188)] and Moffat and coworkers[189)] have prepared 2'- and 3'-halo purine nucleosides (which can be reduced to the deoxynucleosides) via 2',3'-acetoxonium ion intermediates. However, the 2'-halo derivatives are generally only minor products. Extensive work by Goodman and coworkers[190,191,192)] led to a synthesis of 2'-deoxyadenosine from 3'-deoxy-3'-ethylthio-9-β-D-xylofuranosyladenine. Migration of the 3'-ethylthio residue to the 2'-position, via a 2',3'-episulphonium ion, yielded a 2'-ethylthio-3'-chloro derivative. Hydrolysis and subsequent desulphurization gave mixtures of 2'- and 3'-deoxyadenosine with the 2'-isomer predominating. In later work Goodman et al[193)] utilized alkyl 1-thio-α-D-arabinofuranoside derivatives in syntheses of 2'-alkylthio purine nucleosides. This work will be described in greater detail in the Results and Discussion section of this chapter.

2-Deoxyglycosides of simple alcohols are often prepared by dissolution of the 2'-deoxy sugar in alcohol containing acid.194,195,196) This method can, depending on the temperature, concentration of acid and nature of the sugar used, give rise to both furanoside and pyranoside products, usually as mixtures of α and β anomers. Hoffer et al157) and Ness et al197) prepared methyl 2-deoxy-D-ribofuranoside as an anomeric mixture in this manner. The α and β anomers were separated by fractional crystallization as their p-chlorobenzoyl, p-methylbenzoyl or p-nitrobenzoyl esters. Zorbach and coworkers198,199) have used similar procedures in the preparation of methyl furanosides of some 2-deoxyhexose sugars.

The preparation of 2-deoxyglycosides derived from more complex aglycones is generally carried out by condensation of the aglycone with 2-deoxyglycosyl halide derivatives, often in the presence of a metal salt as catalyst or acid acceptor.196) This type of procedure is commonly used in the synthesis of 2'-deoxy nucleosides, as outlined above. Zorbach and coworkers200,201,202) have prepared analogues of the cardiac glycoside digitoxin by condensation of the hydroxy-steroid digitoxigenin with glycopyranosyl halides derived from a series of 2-deoxy sugars. Plim and Šorm203) prepared a symmetrical 1,1'-disaccharide

of 2'-deoxyribose, which had been found as a by-product in 2'-deoxy nucleoside syntheses, by similar procedures.

The mechanism and stereoselectivity of this type of condensation reaction, in the 2-deoxy sugar series, is not well defined. In some cases, reaction of an anomerically pure glycosyl halide with an alcohol[147)] or a hydroxysteroid[200,204)] has been reported to yield anomerically pure glycosidic products in which the aglycone has the opposite configuration to that of the glycosyl halide. In other cases, anomeric mixtures were obtained.[199,202)] In studies on the methanolysis of a series of anomerically pure D-arabinofuranosyl halides having a nonparticipating group at C-2, Glaudemans and Fletcher[205)] have concluded that the anomeric configuration of the products is not directly related to the configuration of the glycosyl halide. However, due to the presence of the 2-substituent in these compounds, this study cannot be directly related to the 2-deoxyglycosyl halides. In the absence of similar studies in this series, it is not possible at present to determine the applicability of this procedure to stereoselective 2-deoxyglycoside synthesis.

Anomeric mixtures of 2-deoxyglycopyranosides have been prepared from the corresponding glycal derivatives by treatment with an alcohol in the presence of an acid

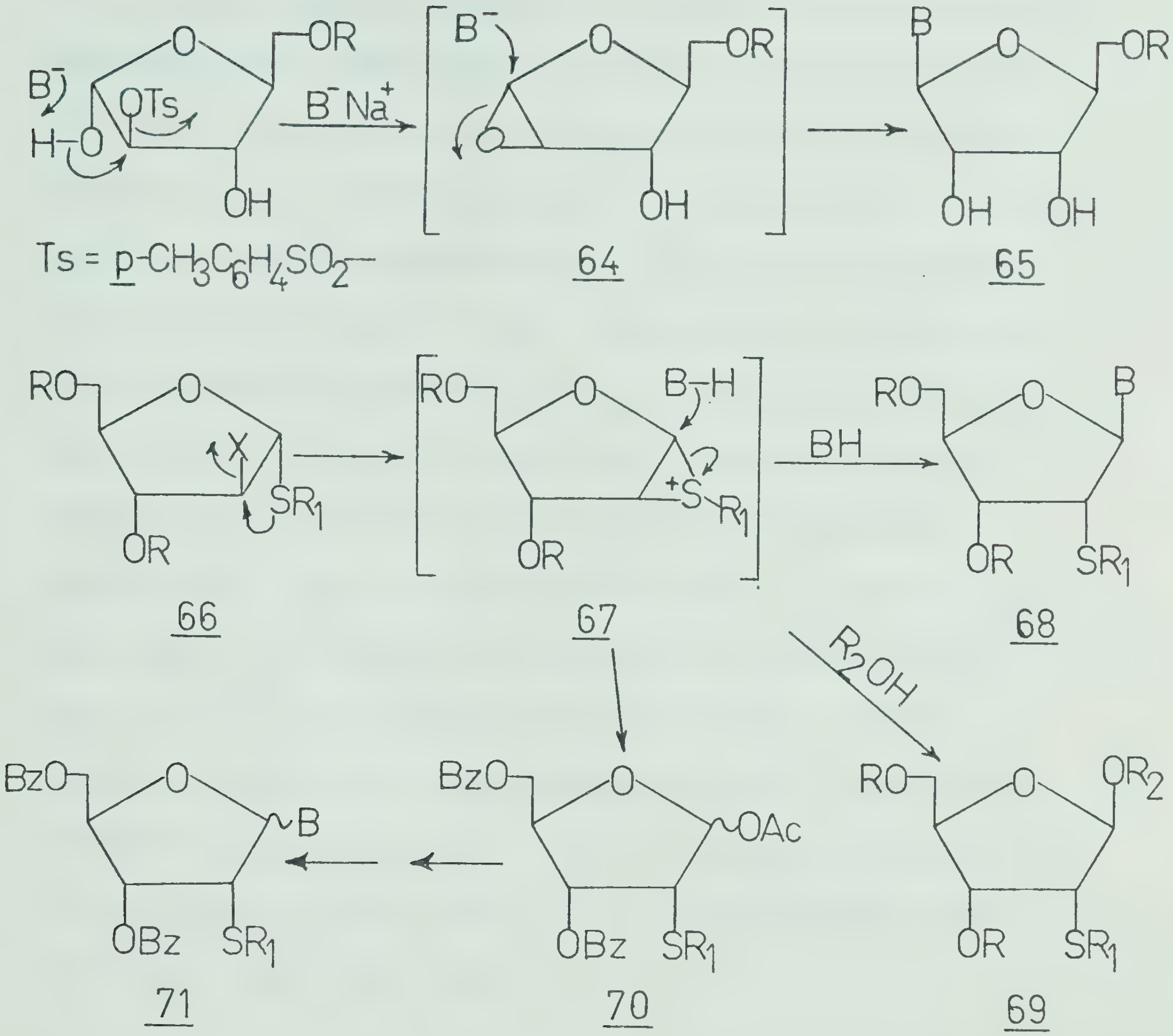
catalyst.[195)] In a related reaction, oxymercuration of glycals in the presence of an alcohol, followed by sodium borohydride reduction of the resulting 2-mercuri glycoside, yields similar products.[206)] However, extension of these procedures to syntheses in the furanoside series has so far been unsuccessful.[207,208)] Ring opening reactions of anomerically pure 2,3-anhydroglycosides can be used in the preparation of 2- and 3-deoxyglycosides.[195)] This procedure and the glycal method have both been utilized in the synthesis of methyl pyranoside derivatives of sugars found in the cardiac glycosides.[209)] In reactions of 2,3-anhydropentofuranoside derivatives the ratio of 2- to 3-substituted products is dependent on the configuration of the alglycone and in some cases the 3-isomers are the only observable products.[210-212)]

RESULTS AND DISCUSSION

The object of the work described below was the development of a stereospecific synthesis of β-2'-deoxynucleosides. 1,2-Anhydro sugars have been used for the stereospecific synthesis of glycosides having a C-1—C-2 trans configuration.[213] Recently the intermediacy of a 1,2-anhydroribofuranose derivative (64) was postulated in the preparation of a a number of β-L-ribonucleosides (65)[214] (see Scheme XI). We had visualized utilization of a 1,2-episulphonium ion (67) in the preparation of 2'-alkylthio nucleosides in an analogous manner. The route envisaged involved preparation of an alkyl or aryl 1-thio-α-D-arabinofuranoside (66) suitably derivatized for generation of the 1,2-episulphonium ion (67). Condensation with purine or pyrimidine bases would then yield 2'-alkylthio-ribonucleosides (68) having the β-anomeric configuration (see Scheme XI). Similar reactions with simple alcohols would be expected to yield β-glycosides (69). Subsequent desulphurization and deblocking would convert these products (68 and 69) into β-2'-deoxy nucleosides and 2-deoxy-β-D-ribofuranoside derivatives, respectively.

In 1959 Goodman and coworkers[215] proposed the use of a 1,2-episulphonium ion intermediate such as

SCHEME XI



X = leaving group

BH = Purine or Pyrimidine base

R_1 = Alkyl or Aryl

R_2 = Alkyl

67 in 2'-deoxynucleoside syntheses. They also reported initial work in the development of a precursor to 67 (66; R = benzoyl, X = hydroxyl, R_1 = ethyl). In a related paper, published in 1971 after this work was commenced, these workers reported the synthesis of some 2'-alkythio nucleosides using compounds related to 66 as intermediates.[193] However, the procedure employed involved conversion of 66 to a 2-alkylthio-1-O-acetyl derivative (70), involving a 1,2-migration of the alkylthio group via the 1,2-episulphonium ion (67). Chlorination of 70 at C-1 followed by condensation with purine bases yielded 2'-alkylthio nucleosides (71) as anomeric mixtures. In one experiment (70, R_1 = benzoyl) nucleosidic products having only the β-configuration were obtained. However, no attempt to convert these products into 2'-deoxy-β-nucleosides was reported. Also no reactions of purine or pyrimidine bases with the 1,2-episulphonium ion precursor (66) were noted.

A. PREPARATION OF PHENYL AND BENZYL 3,5-DI-O-BENZYL-1-THIO-α-D-ARABINOFURANOSIDES.

In order to obtain precursors (66) for the episulphonium ion intermediate 67, we chose 3,5-di-O-substituted-1-thio-α-D-arabinofuranoside derivatives having an unsubstituted 2-hydroxyl group (66, X = OH). Sulphonylation of the 2-hydroxyl group would then yield the desired precursor (66, $X = OSO_2R$). We therefore required an arabinofuranose derivative selectively substituted at either the 1 and 2- or else the 3 and 5-positions. Goodman and coworkers had previously prepared 5-O-benzoyl-1,2-O-isopropylidene-D-arabinofuranose[215)] and 1,3,5-tri-O-benzoyl-D-arabinofuranose.[193)] We chose 1,2-O-isopropylidene-D-arabinofuranose (77) which was prepared using a modification of the procedure reported by Hirst et al[216)] for the synthesis of 5-O-p-toluenesulphonyl-1,2-O-isopropylidene-L-arabinofuranose.

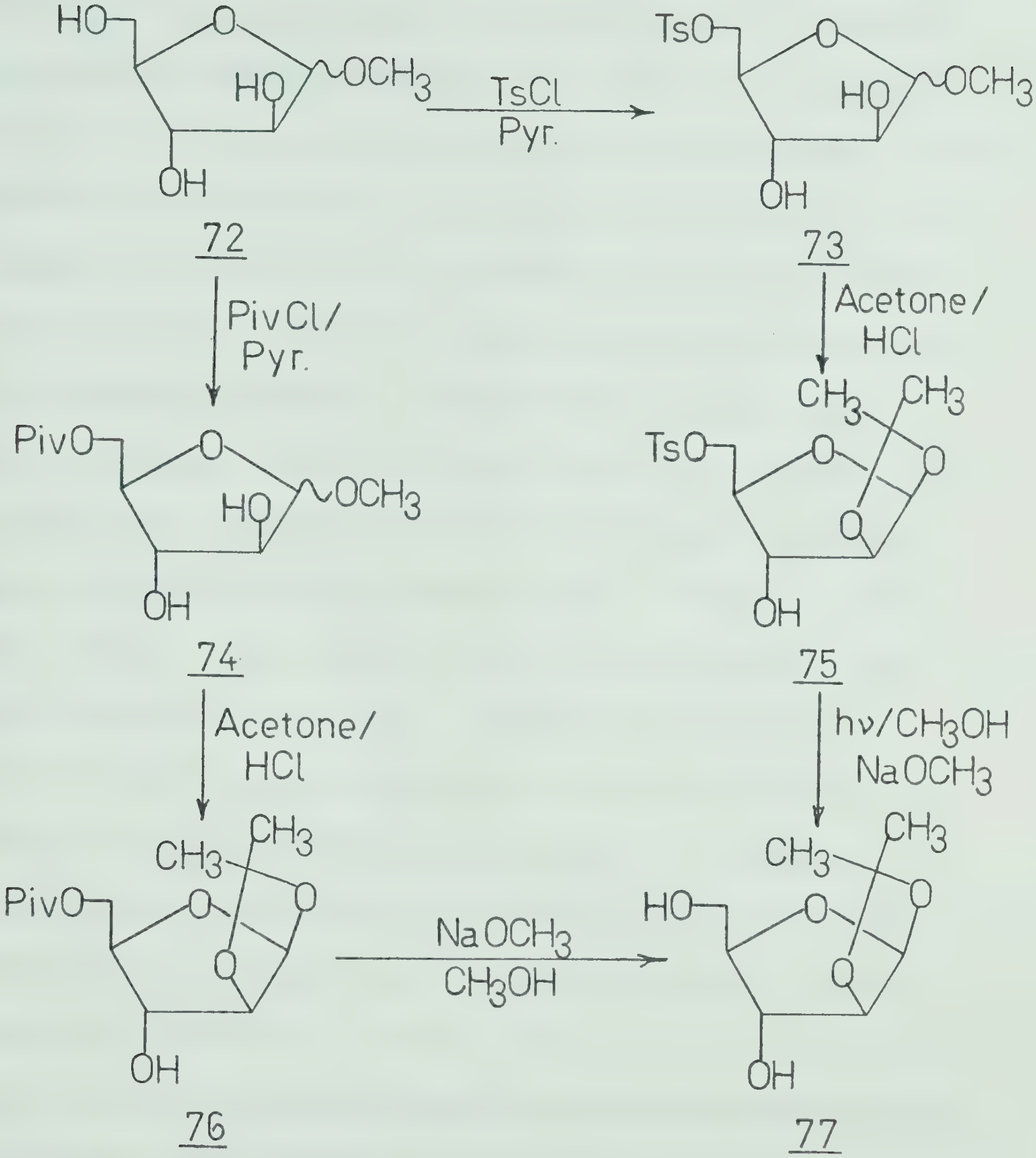
Methyl D-arabinofuranoside (72) was prepared by treatment of D-arabinose with a methanolic solution of hydrogen chloride at room temperature as reported by Ness and Fletcher.[217)] The reaction was quenched, by the addition of silver carbonate, when the optical rotation of the solution had reached a maximum value. Under these conditions, the formation of furanoside

products (72) should be maximized relative to methyl arabinopyranosides.[218)] Upon dissolution of the resulting syrupy 72 in acetone, a small amount (4%) of methyl-β-D-arabinopyranoside crystallized. The remaining crude 72 (95%) was used without further purification.

In order to effect acetonation of 72 at the 1,2-positions, the 5-hydroxyl group must first be blocked so that the sugar is maintained in the furanose ring. Hirst *et al*[216)] accomplished this by selective blocking of the 5-hydroxyl with *p*-toluenesulphonyl chloride in pyridine to yield 73 (see Scheme XII). Investigation of this reaction revealed that di-*O*-*p*-toluenesulphonyl substituted products were formed as well as 73, in a ratio ~1:4.5, respectively. A more convenient procedure was devised involving acylation of the 5-hydroxyl group.

Initial investigations indicated that 72 could be acylated, at low temperature, by pivalyl chloride in pyridine-methylene chloride or pyridine-THF to yield a mixture of methyl 5-*O*-pivalyl-D-arabinofuranoside (74) and dipivalylated products. The ratio of mono to dipivalylation was ~4:1 as estimated by comparison of the *O*-methyl signals in the nmr spectrum of the mixture. Attempts to improve the selectivity of this reaction by variation of temperature and solvent mixtures,

SCHEME XII



Ts = p-$CH_3C_6H_4SO_2$—

Piv = $(CH_3)_3CCO$—

by use of pivalic anhydride in place of pivalyl chloride or by replacement of pyridine with either triethylamine or imidazole were unsuccessful. However, reaction of 72 with only 0.5 equivalents of pivalyl chloride in pyridine-THF at -25°C gave an approximately 1:1 mixture of 72 and 74 containing very little dipivalylated material. The mixture of 72 and 74 was cleanly separated by liquid-liquid extraction, and pyridinium hydrochloride was removed from unreacted 72 by passage through a column of a weakly basic anion exchange resin. In this manner 74 was obtained in 91% yield, based on recovered 72, and contained less than 10% dipivalylated product. This procedure was, however, too tedious for use in repeated large scale preparations of 74. In this case 72 was acylated by 1.1 equivalents of pivalyl chloride in pyridine-methylene chloride at -78°C. Complete separation of unreacted 72, product 74 and dipivalylated products was achieved by a simple series of liquid-liquid extractions. In this manner 74 was obtained, free from dipivalylated material, in 63% yield and a mixture of dipivalylated products was isolated in 18.5% yield.

Acetonation of the 5-*O*-*p*-toluenesulphonyl derivative (73) was performed as described by Hirst *et al*216) (see Scheme XII). Thus treatment of 73

with a solution of hydrogen chloride in dry acetone gave a mixture of products from which the 1,2-O-isopropylidene derivative (75) was isolated in poor yield by column chromatography. Similar reaction with 74 gave 5-O-pivalyl-1,2-O-isopropylidene-D-arabinofuranose (76). Column chromatography of the crude reaction product gave 76 as a syrup in ~60% yield. This product contained a small amount of an impurity that migrated marginally farther than 76 on tlc. Crystallization of 76 depended on the amount of this impurity present and crystalline yields in the range of 35-45% were usually obtained. The position of the pivalyl group at C-5 in 76 was verified by nmr spectroscopy. The spectrum of 76 in DMSO-d_6 displayed a sharp doublet at δ 5.50 which disappeared upon D_2O exchange. This peak can be assigned to the 3-hydroxyl group which is coupled to the C-3 hydrogen. An unsubstituted 5-hydroxyl group would appear as a triplet due to coupling with the two C-5 hydrogens.

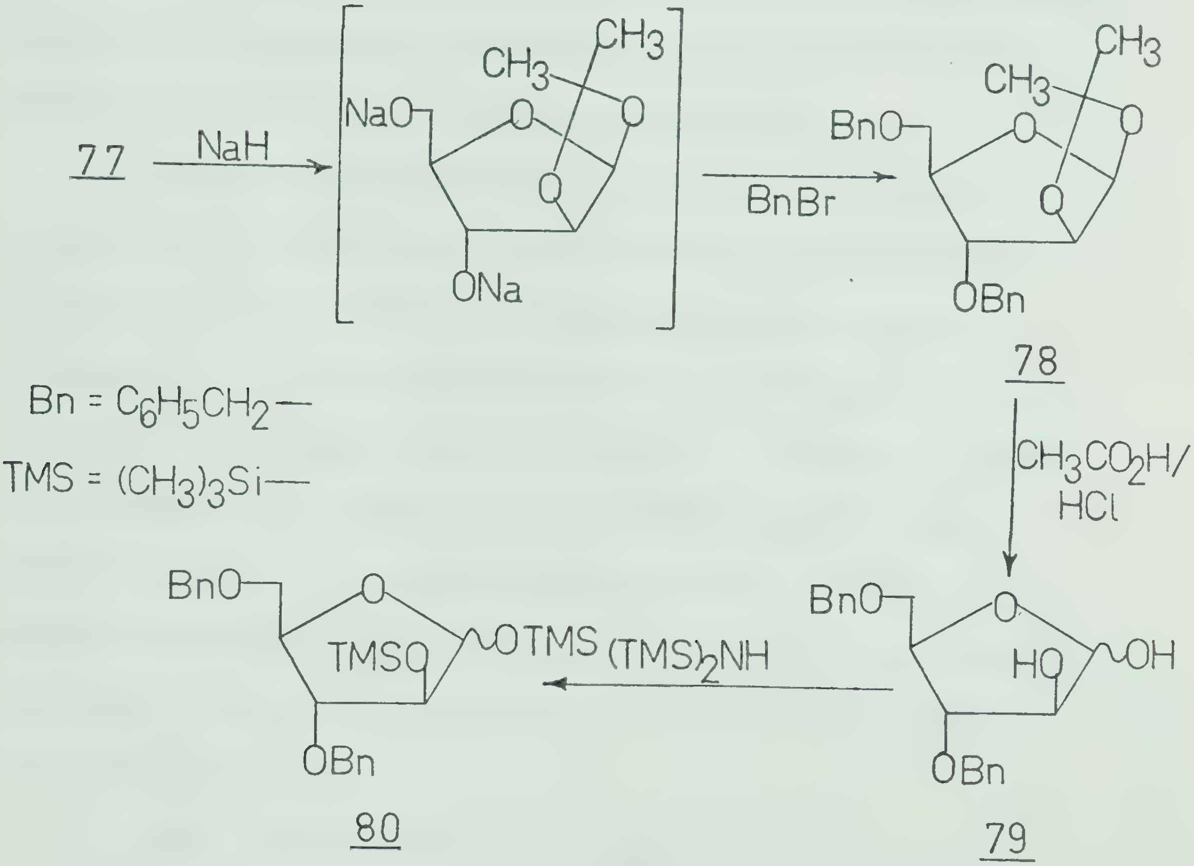
Deacylation of 76 by treatment with sodium methoxide in dry methanol proceeded smoothly to give 1,2-O-isopropylidene-D-arabinofuranose (77) in essentially quantitative yield. This product was characterized by its nmr spectrum in D_2O which showed singlets for the two methyl groups at δ 1.57 and 1.76.

The C-1 and C-2 hydrogens appeared as a pair of doublets at δ 6.21 and 4.89, respectively, each with a coupling constant of 3.8 Hz. There was no observable coupling between H_2 and H_3. Attempts to carry out the deacylation reaction on the crude product obtained in the preparation of 76 were less successful, due to difficulties encountered in purification, and poor yields of 77 were obtained. In one experiment, desulphonylation of 75 was effected by photolysis in methanolic sodium methoxide.[219)] However, the crude reaction product contained a number of minor impurities and crystalline 77 was obtained in poor yield. The procedure required for this reaction was not readily amenable to large scale preparations of 77. For this reason, and also in view of the superior results obtained with pivalylated derivatives, investigation of the use of p-toluenesulphonyl derivatives in the preparation of 77 was not continued.

The next stage in the reaction sequence involved blocking the 3 and 5-hydroxyl groups. The benzyl blocking group was chosen since it would be stable under the various reaction conditions to be used, but would be readily removed by hydrogenolysis. Addition of a DMF solution of 77 to a suspension of sodium hydride in DMF at 0°C yielded a suspension of the disodium salt of 77. A DMF solution of benzyl

bromide was added to this suspension at 0°C to give 3,5-di-O-benzyl-1,2-O-isopropylidene-D-arabinofuranose (78) which was isolated as a yellow syrup in quantitative yield (see Scheme XIII). Temperature control was

SCHEME XIII



Bn = $C_6H_5CH_2$—

TMS = $(CH_3)_3Si$—

important in this reaction. At temperatures below ~20°C formation of the disodium salt was extremely slow, while at temperatures much above 0°C significant orange colouration resulted. No impurity peaks were observed in the nmr spectrum of product 78 and

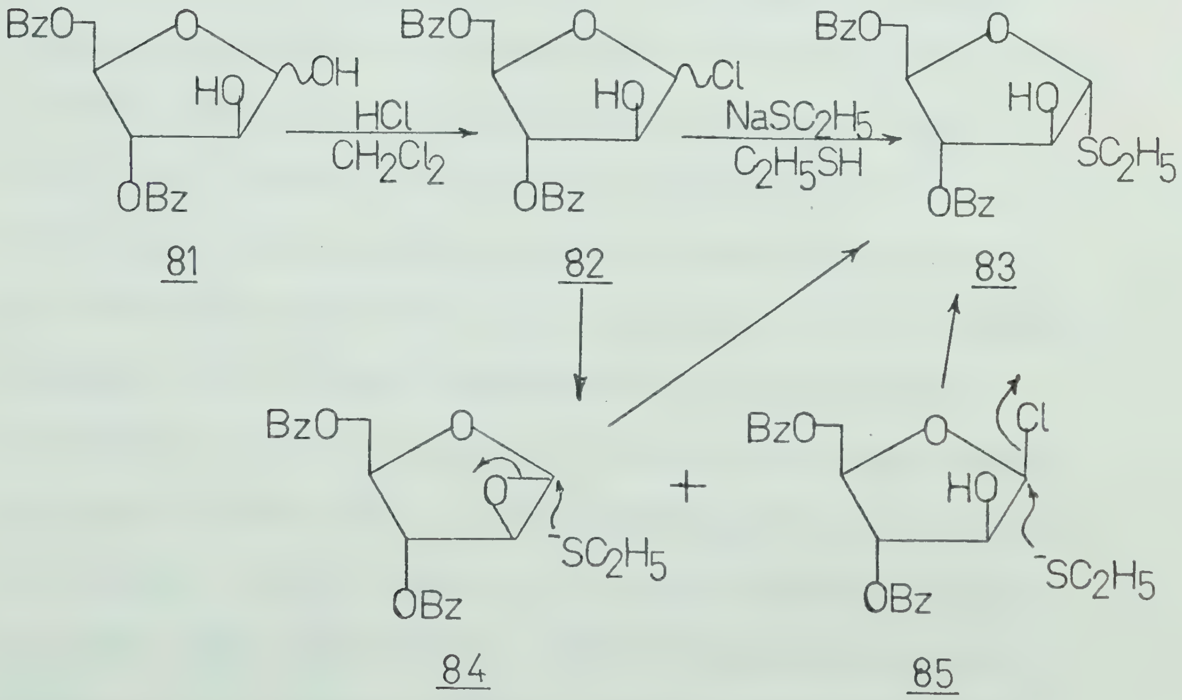
the introduction of the benzyl groups was evidenced by the appearance of a broad singlet at δ 7.30 (aromatic hydrogens) and a pair of singlets at δ 4.54 and 4.56 (methylene groups). The mass spectrum of 78 showed a parent peak at m/e 370.1768 and also a very large peak at m/e 91, corresponding to tropyllium ion, which is derived from the benzyl substituents. All of the benzylated compounds subsequently studied displayed this large tropyllium ion peak.

Quantitative removal of the isopropylidene group from 78 was achieved by acid hydrolysis using conditions previously employed by Goodman and coworkers215) in the deblocking of 3,5-di-O-benzoyl-1,2-O-isopropylidene-D-arabinofuranose. Compound 79 was isolated as a chromatographically homogenous syrup in 97% yield. Trimethylsilylation of 79 using hexamethyldisilazane in dry benzene gave a disubstituted product, whose mass spectrum was compatible with structure 80.

The final stage in the reaction sequence involved introduction of the alkyl- or aryl-thio group at the 1-position of the sugar. In the preparation of ethyl 3,5-di-O-benzoyl-1-thio-α-D-arabinofuranoside (83), Goodman and coworkers215) achieved this by chlorination of 3,5-di-O-benzoyl-D-arabinose (81) and condensation of the resulting 1-chloro derivative (82)

with sodium ethylmercaptide (see Scheme XIV). In this, and similar reactions performed in later work,193) the α-anomer (83) was the highly predominant product. These workers explained this result in terms of participation by the 2-hydroxyl group. They suggested that, under the influence of the strongly basic mercaptide anion, the α-anomer of chloro compound 82 reacts to form a 1,2-anhydro intermediate, 84. The β-anomer 85, having the *C*-1—*C*-2 *cis* configuration, is unable to form this intermediate. Ring opening of 84 by mercaptide ion attack at *C*-1, or simple Sn-2 displacement of chloride by mercaptide in 85, would give only the

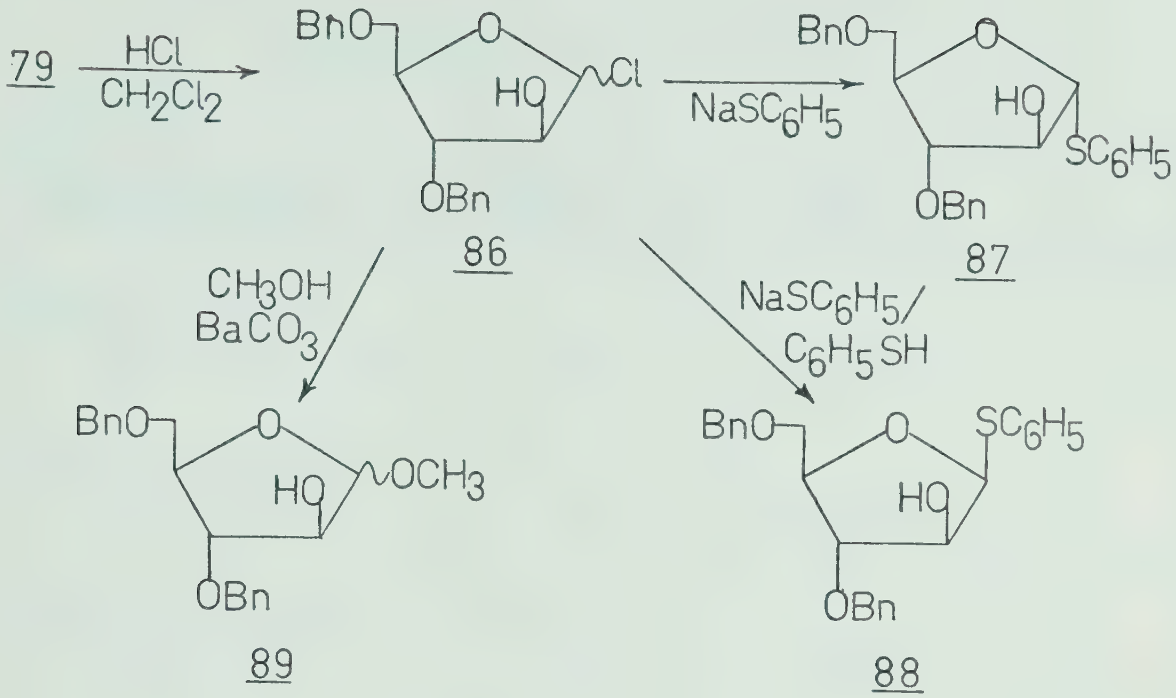
SCHEME XIV



observed α-products. In the present case, problems were encountered in both steps of this reaction sequence.

In early experiments, chlorination was effected by treatment of 79 with dry HCl in methylene chloride over Drierite at 0°C as described by Goodman and coworkers.[215)] The resulting product, which was assumed to be a mixture of the anomeric chlorides 86, was isolated as a yellow syrup after filtration and repeated coevaporation with benzene or toluene to remove excess HCl. Immediate condensation of 86 with a suspension of sodium phenyl mercaptide in dry THF gave low conversion to the α-1-phenylthio product 87 (see Scheme XV). Pure 87 was isolated in 24% yield by column chromatography. A similar condensation carried out in the presence of a two fold excess of benzemethiol gave the anomeric β-product (88) in 19% yield. These two products (87 and 88) migrated differently on tlc and it was observed that each of the above condensations gave mainly one of the anomeric products (87 or 88), depending on the conditions. Assignment of the α and β-configurations to 87 and 88 was made on the basis of their nmr spectra and optical rotations. The H_1 signal in the nmr spectrum of the α-anomer (87) appeared as a broad singlet at δ 5.50. Expansion of this peak gave a poorly resolved triplet

SCHEME XV



with a coupling constant of ~0.65 Hz. In the β-anomer (88) the H_1 signal appeared as a sharp doublet at δ 5.42 with a coupling constant of 3.0 Hz. The very small coupling observed for H_1 in 87 is consistent with the C-1—C-2 *trans* configuration assigned to this compound.[220] Application of Hudsons rules of isorotation[221] to 87 and 88 gives the same assignments and good qualitative agreement with the specific rotations of other alkyl 1-thio-D-arabinofuranosides is observed (see Table V).

In view of the poor yields encountered in the combined chlorination-condensation reaction sequence,

TABLE V

Optical Rotations of Some 1-thio-D-Arabinofuranoside Derivatives

1-Thio-D-arabinofuranoside derivative	Anomeric Configuration	Specific Rotation[a]	Reference
Phenyl 3,5-di-O-benzyl (87)	α	+189.5°	
Phenyl 3,5-di-O-benzyl (88)	β	-118.5°	
Benzyl 3,5-di-O-benzyl (92)	α	+265°	
Ethyl 3,5-di-O-benzoyl	α	+159°	215
Ethyl 5-O-benzoyl	β	- 96.5°	215
Benzyl 3,5-di-O-benzoyl	α	+249°	193

[a] Recorded at 589 nm.

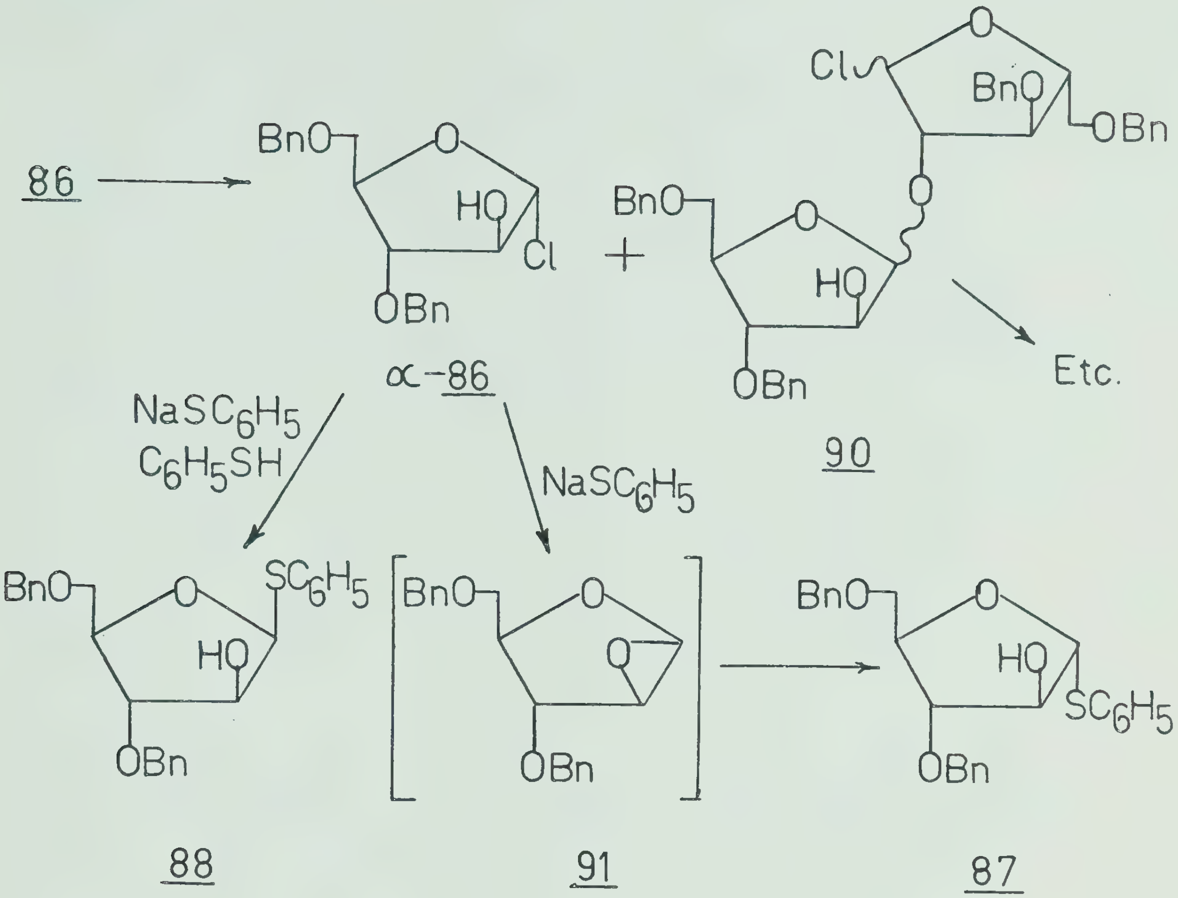
and also of the unexpected preferential formation of the β-anomer under certain conditions, a more detailed investigation of the reaction sequence was undertaken. The chlorination reaction (conversion of 79 to 86) could not be assayed directly by tlc as 86 was rapidly hydrolyzed during the chromatography. However, addition of 86 to a suspension of barium carbonate in methanol gave immediate conversion of 86 to the stable methyl glycoside, 89. Thus, treatment of aliquots of the chlorination reaction in this manner and tlc of the resulting 89 was used to assay the formation of 86. It was found that conversion of 79 to 86 was complete within 15 minutes. However, repeated evaporation during the work up procedure caused conversion of the majority of the chloro product (86) to a material that migrated slightly faster than 89 on tlc. Upon reaction of this mixture with sodium phenyl mercaptide, the remaining 86 was converted to 87. In one experiment, after column chromatographic isolation of 87, continued elution of the column gave partial separation of a number of by-products observed in the reaction. These were tentatively identified as polysaccharides on the basis of their complex nmr spectra and the appearance of unusually high molecular weight fragment ions in their mass spectra. Also, the ultraviolet spectra of these by-products uniformly showed an absorption

maximum at ~ 248 nm, which is characteristic of the phenylthio substituent. However, those by-products eluting more slowly from the column had much reduced absorbances compared with that of 87. This would occur in higher molecular weight di or trisaccharides containing only one phenylthio residue.

The above observations might be rationalized in the following manner. If it is assumed that the 1-chloro product 86 is highly reactive, self condensation could take place to produce 1,2'-linked disaccharides such as 90 during concentration of solutions of 86 (see Scheme XVI). Further condensation could lead to higher polysaccharides and eventually reaction with sodium phenylmercaptide would give the S-phenyl terminated polysaccharide products presumably observed.

As mentioned above, when mixtures of the presumed polysaccharide by-products and 86, from which the majority of the hydrogen chloride had been removed by repeated evaporation, were condensed with sodium phenyl mercaptide the remaining 86 was converted predominantly to the α-1-phenylthio product (87) and only small amounts of the β-anomer (88) were observed on tlc. In contrast, when the condensations were carried out in the presence of excess benzenethiol the remaining 86 was converted mainly to the β-product (88). This is consistant with the assumption that the 1-

SCHEME XVI



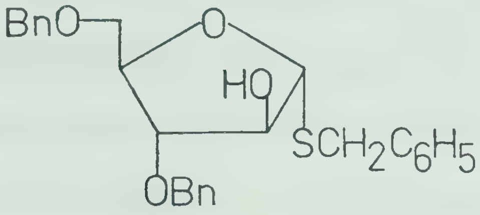
chloro sugar (86) that remains after repeated evaporation is largely the α-anomer (α-86, see Scheme XVI). Reaction of α-86 with sodium phenylmercaptide, in the absence of benzenethiol, could proceed *via* the 1,2-anhydro intermediate (91) to give the α-anomer (87). Alternatively, in the presence of the acidic excess benzenethiol (which is completely soluble in THF, in contrast to the mercaptide salt), simple $S_N{-}^2$ displacement of chloride in α-86 by thiol or mercaptide

to yield the β-1-phenylthio derivative (88) could predominate.

In subsequent preparations, solutions of 86 were not evaporated to dryness during the work-up procedure. In this way little decomposition was observed, but solutions of 86 remained appreciably acidic even after repeated dilution with and partial evaporation of large volumes of benzene. Reaction of such solutions of 86 with sodium phenylmercaptide gave mainly 1-phenylthio products (87 or 88) and only small amounts of presumed polysaccharide by-products. However, tlc comparisons showed that the major product was invariably the β-anomer (88) and only minor amounts of the desired α-anomer (87) were formed. This result could be due to neutralization of the sodium phenylmercaptide by excess acid present and direct displacement of the α-chloro as discussed above. Attempts to neutralize solutions of 86 before use by treatment with barium carbonate were unsuccessful and hydrolysis to 79 as well as polysaccharide by-product formation occurred.

It was considered that use of a more strongly basic mercaptide derivative might enhance formation of the postulated intermediate 1,2-anhydro derivative (91) and therefore give better yields of α-anomeric products. Accordingly, reaction of a solution of chloro-sugar 86

with a suspension of 2 equivalents of sodium benzylmercaptide in THF gave benzyl 3,5-di-O-benzyl-1-thio-α-D-arabinofuranoside (92) in 70% yield after column



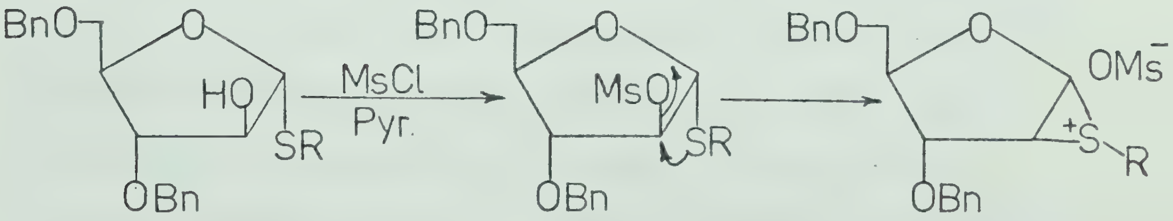
92

chromatography. This material was obtained as an analytically pure syrup and the nmr spectrum indicated the presence of only one anomer. The high positive rotation observed (see Table V) was compatible with assignment of the α-configuration to 92.

B. REACTIONS OF BENZYL AND PHENYL 3,5-DI-O-BENZYL-2-O-METHANESULPHONYL-1-THIO-α-D-ARABINOFURANOSIDE.

In order to induce formation of 1,2-episulphonium ion intermediates such as 67 (Scheme XI), derived from the 1-thio derivatives 87 and 92, the 2-hydroxyl group of these compounds must be functionalized to provide a good leaving group. Accordingly, 87 and 92 were treated with methansulphonyl chloride in pyridine at 0 to -5°C (see Scheme XVII). The 2-O-methanesulphonyl derivatives (93 and 94) were obtained in essentially quantitative yield. The 1-benzylthio product (94) was appreciably more labile than the 1-phenylthio derivative (93) and was invariably contaminated with a trace of material that migrated more slowly on tlc. Both 93 and 94 gave good nmr

SCHEME XVII



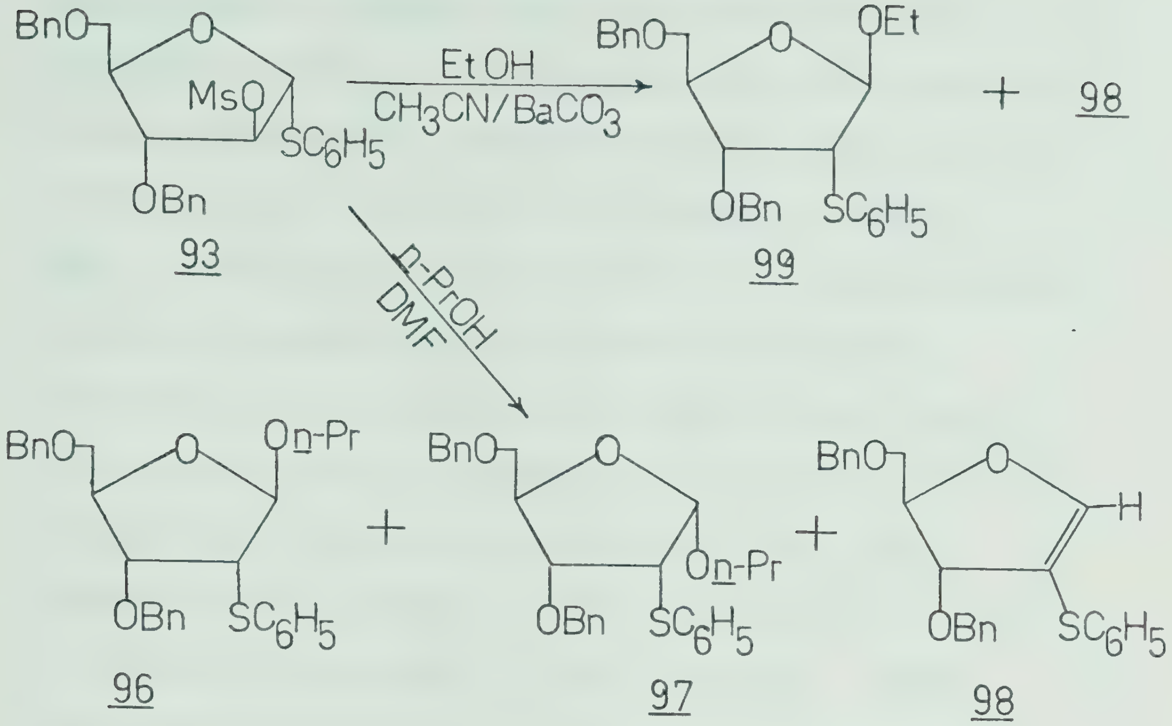
87	R = C_6H_5—	93	R = C_6H_5—	95a
92	R = $C_6H_5CH_2$—	94	R = $C_6H_5CH_2$—	95b

Ms = CH_3SO_2—

spectra and were used immediately in subsequent reactions without further purification. Introduction of the methanesulphonyl group was verified by the appearance of a band at 1182 cm^{-1} in the infrared spectrum of 94.

Formation of 1,2-episulphonium ion intermediates (95) from 93 or 94 *via* displacement of methanesulphonate by the *trans* thio substituent at *C*-1 is now possible. Attack of a suitable nucleophile at *C*-1 of 95 could then produce 2-alkylthio-β-glycosides, as discussed above. Preliminary investigations were carried out with the 1-phenylthio derivative (93). Treatment of 93 with excess *n*-propanol, in either DMF or acetonitrile as solvent at ~60°C, gave moderate conversion to a product that migrated faster than 93 on tlc. The mass spectrum of this product was consistent with the formation of *n*-propyl glycosides (96 and/or 97). Chromatography in a different solvent system revealed the presence of three major components. Preparative tlc resolved isomeric *n*-propyl derivatives which were tentatively identified as *n*-propyl 2-phenylthio-3,5-di-*O*-benzyl-2-deoxy-β and α-D-ribofuranoside (96 and 97, respectively) (see Scheme XVIII). The anomeric hydrogens of 97 and 96 gave rise to doublets in the nmr spectra with coupling constants of 4.75 Hz and

SCHEME XVIII



Et = CH_3CH_2-

n-Pr = $CH_3CH_2CH_2-$

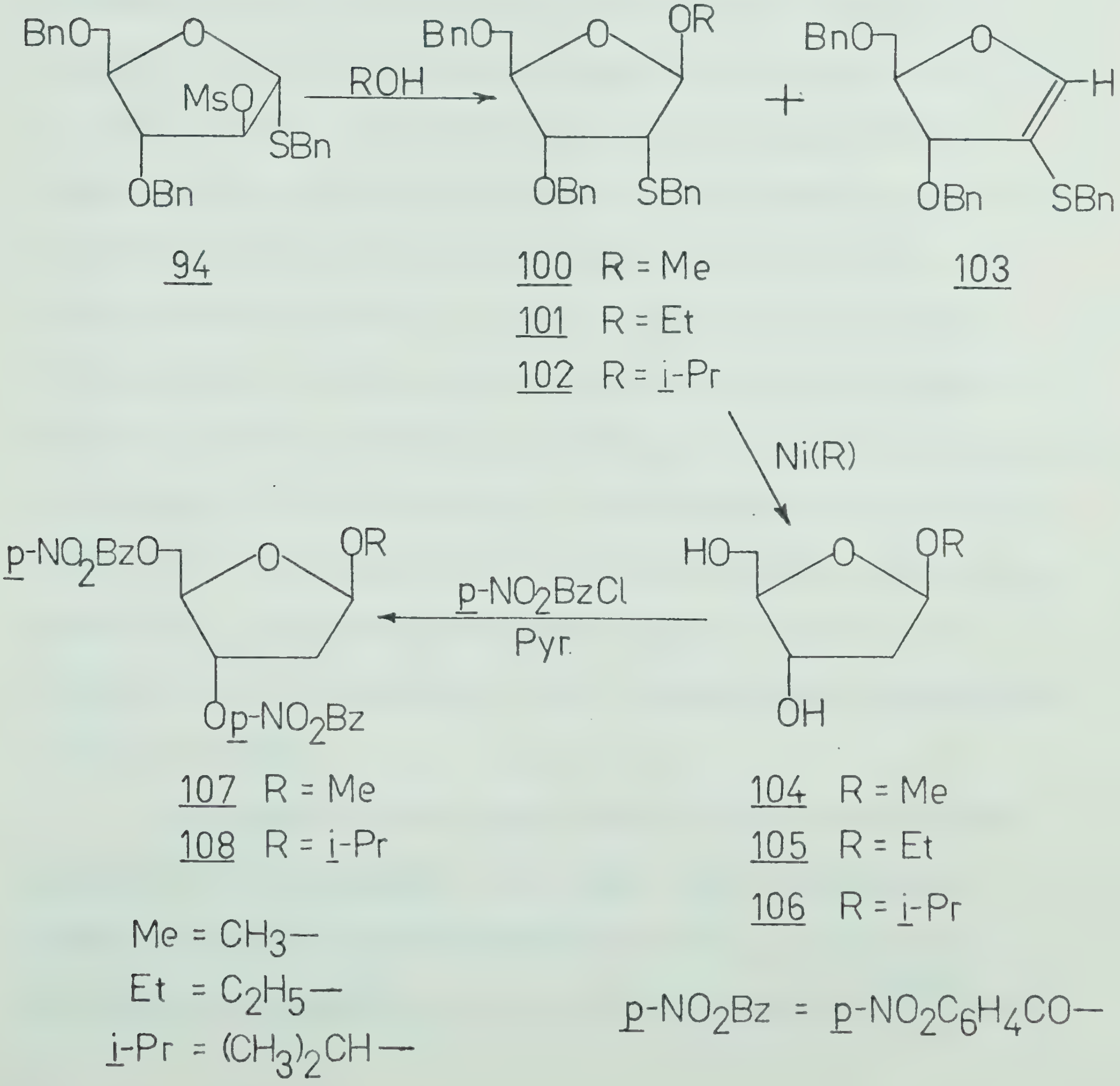
2.3 Hz, respectively. The third component, which was isolated and identified in a subsequent reaction, had mass and nmr spectra consistant with the 2-phenylthioglycal structure, 98. A singlet at δ 6.88 in the nmr spectrum of 98 was assigned to the C-1 hydrogen and indicated that the phenylthio group was shifted to C-2. Compound 98 could arise by deprotonation of the episulphonium ion, 95, at C-2 with concomittant cleavage of the C-1—S bond. Formation of a related pyranoside 2-benzylthioglycal, *via* a 1,2-episulphonium ion, has been reported by Goodman and coworkers.[193)]

Methanesulphonic acid is a by-product in the above reaction and the reaction mixture becomes appreciably acidic. It was possible that formation of the α-anomer (97) resulted from an acid catalyzed anomerization of the initially formed β-anomer (96). Addition of barium carbonate to such reactions prevented accumulation of excess acid and gave colourless reaction products. When 93 was reacted with ethanol in the presence of barium carbonate, the β-glycoside (99) and the glycal (98) were the observed major products. The corresponding α-anomer of 99 was not detected in this reaction. These experiments indicated that stereospecific synthesis of 2-phenylthio-β-glycosides was possible by this route. Due to the difficulties encountered in the preparation of the 1-phenylthio derivative (87), no further reactions in this series were explored.

Reaction of the 1-benzylthio derivative (94) with ten equivalents of ethanol in acetonitrile at 60°C in the presence of barium carbonate gave good conversion to a product that migrated faster than 94 on tlc. Reaction was complete within 17 hours and preparative tlc gave a 73% yield of syrupy ethyl 2-benzylthio-3,5-di-O-benzyl-2-deoxy-β-D-ribofuranoside (101). Similar reactions with methanol and iso-propanol gave the methyl and iso-propyl β-D-

glycosides (100 and 102) in 80.5 and 73% yields, respectively (see Scheme XIX). In each case a minor product was observed in the reactions which migrated marginally faster than the glycosidic products on tlc. In one instance this product was isolated in ~1% yield and was subsequently identified as the 2-benzylthioglycal (103).

SCHEME XIX



The nmr spectra of 100-102 were sharply defined and indicated that these glycosidic products were anomerically pure. Introduction of the aglycone (methyl, ethyl or iso-propyl) was evident from the appearance of characteristic signals for these substituents and loss of the mesyl function (singlet at δ ~2.8) was apparent. The position of the benzylthio substituent at C-2 was indicated by the marked (~0.8 ppm) upfield shift of the signal assigned to the C-2 hydrogen. The C-1 hydrogen of 100, 101 and 102 appeared as a doublet at δ 4.96, 5.08 and 5.22 having coupling constants of 2.8, 3.2 and 3.5 Hz, respectively. The mass spectrum of 100, 101 and 102 each contained a low intensity parent ion whose exact mass corresponded to introduction of the respective methyl, ethyl or iso-propyl aglycone. Also present in each spectrum was a moderate intensity peak at m/e 418. This peak which corresponds to the parent ion minus the protonated C-1 substituent was uniformly observed in the spectra of all 2-benzylthio derivatives prepared. A corresponding peak at m/e 404 was also observed in the spectra of the 2-phenylthio derivatives (96, 97 and 99).

The anomeric configuration of the 2-benzylthio glycosides was somewhat uncertain. The relatively small coupling constants observed for H_1 in the nmr spectra are suggestive of the β-configuration,[220)] but

the α-configuration cannot be ruled out. The specific rotations of 100, 101 and 102, recorded at the sodium D-line, were +57°, +41.5° and +41.9°, respectively. These values are suggestive of the α-configuration,[221)] but conclusions are tenuous in the absence of the opposite anomer for comparison. Conclusive proof that 100, 101 and 102 have the β-configuration was obtained by conversion to known derivatives of 2-deoxy-β-D-*erythro*-pentofuranoside (2-deoxy-β-D-ribofuranoside).

Complete desulphurization with concomitant debenzylation was achieved by heating 100, 101 or 102 with an 11 to 16 fold excess of Raney Nickel in refluxing ethanol (see Scheme XIX). Rapid and essentially quantitative conversion to the alkyl 2-deoxy-β-D-ribofuranosides occurred and 104, 105 and 106 were isolated in 99, 93 and 100% yields, respectively. These crude products contained less than 5% of material which was incompletely debenzylated. The nmr spectra in DMSO-d_6 showed loss of all three benzyl residues and the appearance of a doublet and triplet, which disappeared after D_2O exchange, confirmed the presence of the 3 and 5-hydroxyl groups. Desulphurization to give the 2-deoxy derivatives was evidenced by the appearance of characteristic multiplets at δ ~1.9 for the two *C*-2 hydrogens and also by the appearance of the H_1 resonance as a doublet of doublets (104 and 105)

or a triplet (106) due to coupling with the C-2 hydrogens.

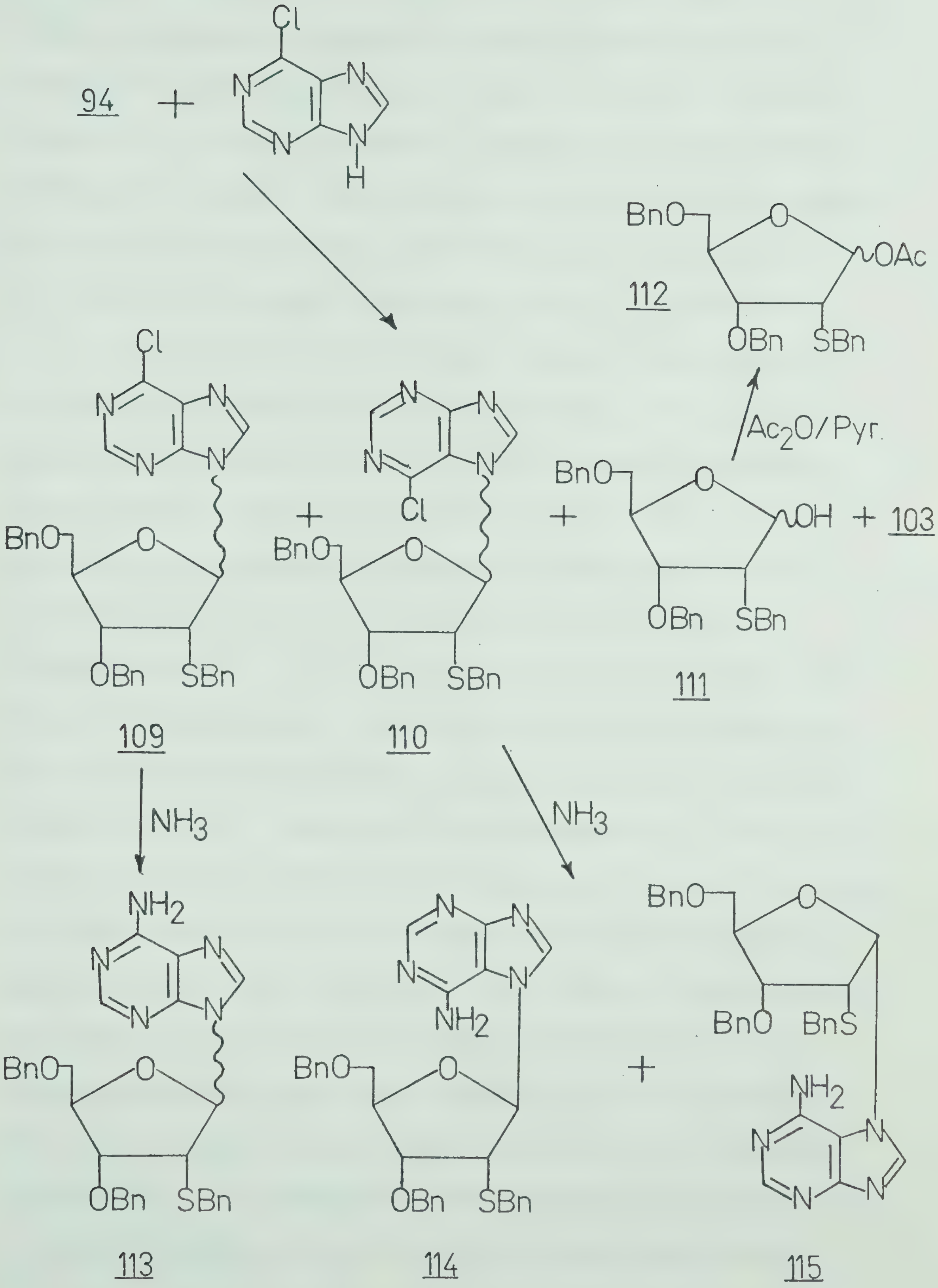
The ethyl 2-deoxyglycoside (105) had $[\alpha]_D^{22}$ -63.7°. This is in contrast to the value ($[\alpha]_D^{20}$ + 48.8°) reported[222)] for the α-anomer of 105 which supports the β-glycosyl configuration of 105 and therefore also of 101. The methyl and iso-propyl 2-deoxyglycosides (104 and 106) were converted to their crystalline 3,5-di-O-p-nitrobenzoyl derivatives (107 and 108). The melting points and optical rotations observed for these compounds were in excellent agreement (see Experimental Section) with those reported by Ness et al[197)] for β-anomers prepared from 2-deoxy-D-ribose, and therefore the β-configuration of the glycosides 100, 102, 104 and 106 is conclusively demonstrated. The above condensation and desulphurization procedure represents a simple, relatively efficient and stereospecific synthesis of alkyl 2-deoxy-β-D-ribofuranosides in two steps from benzyl 2-O-methanesulphonyl-3,5-di-O-benzyl-1-thio-α-D-arabinofuranoside (92).

Having shown that stereospecific attack of a nucleophile at C-1 of the episulphonium ion intermediate (95b) was possible, extension of this type of reaction to the synthesis of 2'-deoxy nucleosides was explored. Reaction of 94 with 2,4-O-bis(trimethyl-

silyl)thymine in acetonitrile or toluene gave very low conversion to a product which had uv spectra consistant with those of a thymine nucleoside. The major product of this reaction was the glycal 103. The reaction of 94 with 6-chloropurine was much more promising.

Treatment of 94 with 2 equivalents of 6-chloropurine in acetonitrile at 60°C gave moderate conversion to the nucleosidic products 109 and 110 as well as the 2-benzylthio glycal (103) and a sugar hydrolysis product (111) (see Scheme XX). The reaction was carried out using barium carbonate as an acid acceptor. Unreacted 6-chloropurine was removed by extraction into saturated sodium bicarbonate solution and the remaining complex mixture of products was separated by a combination of column chromatography and preparative tlc. In this manner, the 9 and 7-glycosyl purine derivatives (109 and 110) were isolated in 25 and 11% yields, respectively, and the glycal (103) was obtained in 20.5% yield. The glycal was identified on the basis of its nmr and mass spectra. The nmr spectrum was fully interpreted and a narrow doublet at δ 6.38 with a coupling constant of 0.9 Hz was assigned to the C-1 hydrogen. A fourth component of the reaction mixture, which was only narrowly separated from 109 on preparative tlc, was obtained in

SCHEME XX


94
+
Cl
N
H
112
BnO
OAc
OBn
SBn
Ac$_2$O/Pyr.
OH
+ 103
109
110
111
NH$_3$
NH$_3$
NH$_2$
BnS
113
114
115

~31% yield and was tentatively identified as 2-benzylthio-3,5-di-O-benzyl-2-deoxy-D-arabinose (111). This product presumably arises by hydrolysis of the episulphonium ion intermediate (95b) by water. Acetylation of 111 gave an anomeric mixture of the 1-O-acetyl derivative (112). The nmr spectrum of crude 112 showed two acetyl methyl peaks, and two narrow doublets at δ 6.27 and 6.18 were assigned to the C-1 hydrogen.

Structure proofs of 109 and 110 were based initially on their spectral properties. The mass spectra were consistent with the proposed structures and accurate mass determination of the parent ions verified the molecular formulae. Comparison of the uv spectra with those of known 7 and 9-alkylated-6-chloropurines[223,224,225)] indicated 109 and 110 to be 9 and 7-glycosylated, respectively. The nmr spectra were cleanly resolved and in agreement with the structures assigned. However, the appearance of a second series of small peaks for the C-2, C-8 and C-1' hydrogens in each spectrum suggested that both 109 and 110 were mixtures of α and β-anomers. The ratio of the anomers was ~9:1 in 109 and ~4:1 in 110. Since β-anomeric stereospecificity had been expected in this reaction, the major anomers were tentatively assigned the β-configuration. The structures and anomeric configurations of 109 and 110 were confirmed by conversion into 2'-

deoxy-9 and 7-*D*-ribofuranosyladenine derivatives (see Scheme XX and XXI).

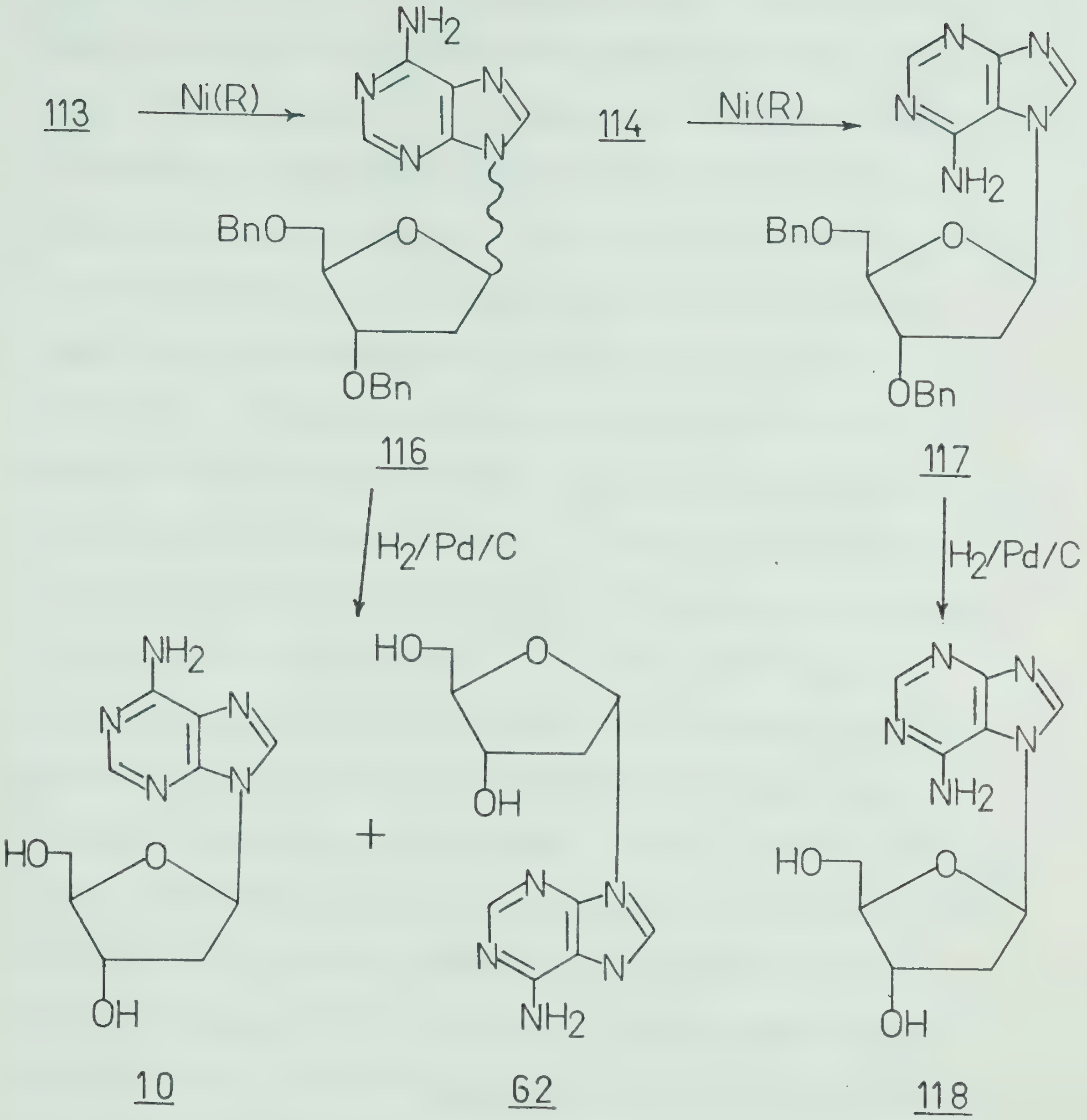
Essentially quantitative replacement of the 6-chloro groups of *109* and *110* was effected by treatment with liquid ammonia.226) 9-(2-Benzylthio-3,5-di-*O*-benzyl-2-deoxy-α,β-*D*-ribofuranosyl)adenine (*113*) was obtained in 98% crude yield. A purified sample of this material was isolated as a solid foam in 90% yield by preparative tlc. The product of amination of *110* displayed two components which were separated by preparative tlc. The major, faster migrating component was isolated in 69.5% yield and crystallization gave 50.5% of 7-(2-benzylthio-3,5-di-*O*-benzyl-2-deoxy-β-*D*-ribofuranosyl)adenine (*114*). All of this material was subsequently used in attempted desulphurization reactions and an analytically pure sample of *114* was not obtained. The minor component of the amination of *110* was isolated in 15% yield as an amorphous solid which gave 12% of the analytically pure α-anomer (*115*) upon crystallization. The uv spectra of these products confirmed the position of glycosylation. The 7-isomers (*114* and *115*) had very broad, characteristic absorptions around 270-275 nm225) whereas the 9-isomer (*113*) showed a relatively narrow absorption at ~259 nm.223) Although the extinction coefficients of the 7-isomers (114 and 115) were appreciably reduced from that re-

ported for 7-methyladenine,[225)] they were in very good agreement with those reported by Goodman and coworkers[193)] for two 7-ribofuranosyladenine derivatives.

Desulphurization and debenzylation of 113 and 114 gave the 2'-deoxy nucleosides 10, 62 and 118 (see Scheme XXI). Attempts to effect these transformations by refluxing with a suspension of Raney Nickel in ethanol (the procedure which was highly effective in the desulphurization and debenzylation of the glycosides 100, 101 and 102) failed completely. This reaction was performed under both neutral and basic conditions and with varying weight ratios of Raney Nickel. When a small excess of Raney Nickel was used, minimal desulphurization of 113 occurred to give the 3',5'-di-O-benzyl-2'-deoxy derivative, 116. Addition of further Raney Nickel resulted in almost complete loss of uv absorption due to the adenine chromophore and appearance of many non-uv absorbing by-products on tlc. Limited success in the desulphurizations of 113 and 114 was achieved, however, when a modified procedure reported by Goodman and coworkers[192,193)] was employed.

A solution of 113 in DMF was treated with a 15 fold excess of Raney Nickel under an atmosphere of hydrogen. When this mixture was heated to ~100°C with an infrared lamp, rapid conversion of 113 to a product

SCHEME XXI



that migrated slightly slower than 113 on tlc, occurred. This was separated from a small amount of unreacted 113 and other minor impurities by preparative tlc to yield 40% of 9-(3,5-di-O-benzyl-2-deoxy-D-ribofuranosyl)adenine (116) which was largely the β-anomer. No

peaks for the α-anomer were observed in the nmr spectrum of 116 and the appearance of a two proton multiplet at δ ~2.65 indicated desulphurization to the 2'-deoxy derivative. Retention of the 3' and 5'-O-benzyl groups was apparent from both the nmr and mass spectra of 116. This is in contrast to the desulphurizations of 100, 101 and 102 in which cases rapid and concomitant debenzylation also occurred. No debenzylation was observed in the present system even when the reaction was performed for extended periods of time. Complete debenzylation of 116 was accomplished by hydrogenolysis using 10% palladium on charcoal.227) The resulting mixture of the β and α-anomers of 9-(2-deoxy-D-erythro-pentofuranosyl)adenine (10 and 62) was separated by column chromatography on Dowex 1-X8(OH^-) anion exchange resin.128) The major β-anomer (10) was crystallized in 56% yield and was identical with authentic 2'-deoxyadenosine in all respects. A very small amount (2.2%) of the α-anomer (62) was obtained as a microcrystalline solid. This material was chromatographically identical with authentic 62 and the melting point of 209-212°C was not depressed on admixture with authentic 62, which has a reported228) melting point of 212-213.5°C.

Application of the procedure used above to the

desulphurization of **114** was less successful. After preparative tlc, 7-(3,5-di-*O*-benzyl-2-deoxy-β-*D*-ribofuranosyl)adenine (**117**) was obtained in but 20% yield and 18% of unreacted **114** was recovered. Debenzylation of **117** was effected as described above and 7-(2-deoxy-β-*D*-*erythro*-pentofuranosyl)adenine (118) was obtained in 82% yield. This material migrated much more slowly than the corresponding 9-isomers (**10** and **62**) on tlc and had nmr and mass spectra consistent with the proposed structure. Compound **118** was extremely acid labile and, in contrast to the 9-isomers (**10** and **62**), its uv spectrum in 0.1 *N* HCl corresponded to the characteristic absorption of adenine. The spectra in alcohol and base are, however, consistent with those reported for other 7-glycosyladenine derivatives.193) The β-configuration was initially assigned to **118** and this was supported by comparison with the corresponding α-anomer (**63**) which was reported by Ness and Fletcher.158) These workers report mp 153-156°C and $[\alpha]_D$ +7 for **63**. These values may be contrasted with the mp 211-212.5°C and $[\alpha]_D$ ~-74° presently observed for **118**. Therefore **63** and **118** must be anomerically related compounds and consequently **118** must have the β-configuration.

In conclusion, the present work has demonstrated the viability of the use of 1,2-episulphonium ion

intermediates related to 95 in the synthesis of 2-alkylthio glycosides. Reactions with alcohols can be effected stereospecifically and efficient preparations of alkyl 2-deoxy-β-D-ribofuranosides have been developed. The reaction with 6-chloropurine, although not stereospecific, gave a high degree of stereoselectivity and β-anomers were the major products. Transformation of the resulting 2'-alkylthio nucleosides into 2'-deoxy nucleosides is dependent on the rather unpredictable efficiency of the desulphurization process using Raney Nickel and low overall yields of 2'-deoxy nucleosides were obtained. This work has resulted, however, in the first reported synthesis of 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)adenine (118), which is the 7-isomer of naturally occurring 2'-deoxyadenosine (10).

EXPERIMENTAL

A. GENERAL PROCEDURES

Melting points were determined on a Reichert microstage apparatus and are uncorrected. Nuclear magnetic resonance (nmr) spectra were recorded on Varian HA-100 or A-60 spectrometers with TMS as the internal standard unless otherwise stated. Peak assignments were verified and, in some cases, individual coupling constants were determined by spin-spin decoupling experiments. Ultraviolet (uv) spectra were recorded on Cary 15 or Pye Unicam SP 1700 spectrometers. Solutions were prepared by diluting a 1 ml or, in cases where solubility problems were encountered, a 5 ml sample of an accurately determined stock solution in MeOH to 10 ml with MeOH, 0.1 N HCl or 0.1 N NaOH. Optical rotations were determined on a Perkin-Elmer model 141 polarimeter using a 10 cm, 1 ml microcell. Mass spectra (MS) were determined by the mass spectrometry laboratory of this department on AEI MS-2, MS-9 or MS-50 instruments at 70 eV. Sample introduction was *via* a direct probe at temperatures between 120 and 230°C. Relative intensities and identities of mass spectral ions are quoted in parentheses following the m/e value. All benzylated compounds had a very high intensity peak at m/e 91 and in these cases a lower intensity peak was chosen

as the mass spectral base peak. Elemental analyses were determined by the microanalytical laboratory of this department.

Thin layer chromatography (tlc) was performed on Eastman chromatogram sheets (silica gel No. 13181, indicator No. 6060) or on glass plates coated with Merck silica gel GF-254. Developed chromatograms were evaluated under uv (2537 Å) light or were sprayed with a 5% solution of H_2SO_4 in EtOH and heated on a hot plate. Preparative tlc was performed on glass plates coated with Merck silica gel PF-254. Product containing bands were removed from the plates, packed in short columns and the product eluted from the silica gel using a suitable solvent mixture. Silica gel column chromatography was performed on J. T. Baker No. 3405 silica gel or Gebr. Herrmann Kieselgel. Evaporations were carried out using a Büchler rotating evaporator with a Dry Ice cooled Dewar condenser under aspirator or oil pump vacuum, at 40°C or less. The term coevaporation refers to the process of dissolution in and evaporation of the solvent indicated. The weights of syrupy products for which a percentage yield is quoted have been corrected for the weight of solvent remaining in the syrup, which was estimated from the nmr spectrum.

Pyridine and tetrahydrofuran (THF) were dried

by refluxing over and then distillation from calcium hydride. Dimethylformamide (DMF) was distilled at reduced pressure from either phosphorus pentoxide or barium oxide. Acetonitrile was distilled from phosphorous pentoxide and methanol was fractionally distilled. Acetone was refluxed over potassium permanganate and then twice distilled from anhydrous calcium sulphate (Drierite). The above solvents were stored over Linde 4A molecular sieves (dried at 200°C). Benzene and toluene were dried over sodium wire and methylene chloride was distilled and stored over calcium chloride. All other solvents used were of reagent purity and were used without purification. Pivalyl chloride, methanesulphonyl chloride and benzyl bromide were purified by distillation prior to use. Hydrogen chloride was dried by passage through concentrated sulphuric acid and then Drierite.

Hydrogenations were carried out at room temperature in a stainless steel bomb filled with hydrogen at the pressure specified and were stirred magnetically. The catalyst used for hydrogenations was Matheson, Coleman and Bell 10% palladium on charcoal. Raney Active Nickel Catalyst (No. 28) was purchased from W. R. Grace and Co.

B. SYNTHESES

Methyl D-Arabinofuranoside (72).

To 100 g(0.67 moles) of D-arabinose suspended in 1.5 ℓ of dry MeOH was added 500 ml of MeOH containing 9.0 g (0.26 moles) of dry HCl. The resulting mixture was stirred magnetically and an homogenous solution was produced after 7 hr. After 23 hr, when the optical rotation of the solution had reached a maximum value, 50 g of Ag_2CO_3 was added and stirring was continued for a further 2 hr. The mixture was filtered through Celite, the filtrate was evaporated and the residue was dissolved in MeOH. Celite and charcoal were added to this solution and the resulting mixture was stirred overnight. Filtration through Celite removed remaining silver salts and the filtrate was evaporated to yield crude 72 as a yellow syrup. This material was dissolved in 500 ml of acetone and, after seeding, 4.30 g (4%) of methyl β-D-arabinopyranoside crystallized as yellow granules: mp 166-168°C; $[\alpha]_D^{24}$ -221.5° (c 1, MeOH). Reported:218) mp 169°C; $[\alpha]_D$ -242°. The mother liquor from the crystallization was evaporated to yield 103.7 g (95%) of crude 72 as a pale yellow syrup: $[\alpha]_D^{24}$ +53.8° (c 2.07, MeOH); nmr (D_2O, TMS external) δ 3.76 (s, 3, OCH_3), 5.21 (d, J_{1-2} ~ 3.8 Hz, β-H_1), 5.24 (d, J_{1-2} ~

1.2 Hz, α-H_1); the ratio of α to β-anomers was ~2:1, respectively, as determined from the nmr spectrum.

Methyl 5-*O*-*p*-toluenesulphonyl-*D*-arabinofuranoside (73) and 5-*O*-*p*-Toluenesulphonyl-1,2-*O*-isopropylidene-*D*-arabinofuranose (75).

The title compounds were prepared from 72 in the manner described by Hirst *et al*.[216] Crude 75 was obtained as an orange syrup. Purification of this material by chromatography on a silica gel column developed with $CHCl_3$—MeOH (96:4) gave an overall yield of 19% of 75 as a colourless solid. Recrystallization from $CHCl_3$—pentane yielded colourless needles of 75: mp 124-126°C; nmr ($CDCl_3$—D_2O) δ 1.33, 1.42 (s, s; 3, 3, $C(CH_3)_2$), 2.50 (s, 3, $C_6H_4CH_3$), 4.2-4.35 (m, 4, H_3, H_4, H_5, $H_{5'}$), 4.59 (d, J_{2-1} ~ 3.8 Hz, 1, H_2), 5.97 (d, J_{1-2} ~ 3.8 Hz, 1, H_1), 7.64 (AB quartet, 4, C_6H_4). Reported:[216] mp 130°C.

Anal. Calcd for $C_{15}H_{20}O_7S$: *C*, 52.31, *H*, 5.85; *S*, 9.31. Found: *C*, 51.95; *H*, 5.86; S, 9.37.

Methyl 5-*O*-Pivalyl-*D*-arabinofuranoside (74).

(a) *Using 1.1 equivalents of pivalyl chloride*: A solution of 20.75 g (0.126 moles) of 72 in 100 ml of

dry pyridine and 300 ml of dry CH_2Cl_2 was stirred magnetically and cooled to -78°C. To this solution was added dropwise, over a period of 2.5 hr, a solution of 16.60 g (0.138 moles) of pivalyl chloride in 100 ml of CH_2Cl_2. The resulting mixture was stirred a further 0.5 hr at -78°C and was then poured into 500 ml of vigorously stirred H_2O. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 100 ml). The combined organic phase was evaporated and the residue was coevaporated with toluene (3 x 100 ml) to yield a yellow syrup of crude 74. The syrup was dissolved in 300 ml of pentane—Et_2O (2:1) and the resulting solution was extracted with H_2O—MeOH (7:3, 100 ml). The aqueous phase was extracted with pentane—Et_2O (2:1, 60 ml) and the organic extract was combined with the original pentane—Et_2O solution. The combined organic phase was then subjected to two further repetitions of this procedure. The resulting organic solution contained only dipivalylated methyl D-arabinofuranoside derivatives and was evaporated. Coevaporation of the residue with acetone (2 x 100 ml) yielded 7.86 g (18.5%) of the dipivalylated material as a pale yellow syrup.

The combined aqueous phase from the above extraction procedure was diluted with 100 ml of H_2O and extracted with CH_2Cl_2 (8 x 75 ml). The combined

organic extracts were evaporated and the residue was coevaporated with acetone (3 x 100 ml) to yield 19.84 g (63%) of chromatographically homogenous (tlc, $CHCl_3$—MeOH, 95:5, R_f ~ 0.2) $\underline{74}$: $[\alpha]_{\underline{\underline{D}}}^{22}$ +42.6° ($\underline{c}$ 1.9, $CHCl_3$); nmr ($CDCl_3$—D_2O) δ 1.24 (s, 9, $C(\underline{CH_3})_3$) 3.35, 3.38 (s, s, 3, α,β-$O\underline{CH_3}$), 4.84 (d, $\underline{J}_{1-2}$ ~ 1.7 Hz, α-$\underline{H}_1$), 5.05 (d, $\underline{J}_{1-2}$ ~ 4.3 Hz, β-$\underline{H}_1$), (DMSO-$\underline{d}_6$) δ 5.24, 5.35 (d, d, $\underline{J}$ = 5.1, 4.8 Hz, 2, 2-$\underline{OH}$, 3-$\underline{OH}$); ms m/e 217.1076 (6.9, M-OCH_3, calcd for $C_{10}H_{17}O_5$: 217.1076), 57 (100, $(CH_3)_3C^+$).

(b) $\underline{\text{Using 0.51 equivalents of pivalyl chloride}}$: A solution of 21.8 g (0.133 moles) of $\underline{72}$ in 100 ml of dry pyridine and 250 ml of dry THF was stirred magnetically and cooled to -25°C. To this solution, a solution of 8.45 g (0.070 moles) of pivalyl chloride in dry THF was added dropwise over a period of 2.5 hr. The resulting suspension was allowed to warm to room temperature and was stirred for a further 1 hr. The reaction mixture was then poured into 500 ml of H_2O and 200 ml of Et_2O was added. The organic phase was separated and the aqueous phase was extracted with Et_2O (3 x 200 ml). The combined organic phase was washed with H_2O (100 ml) and the aqueous wash was extracted with Et_2O (50 ml). The combined aqueous phase was evaporated and an aqueous solution of the residue was applied to a column (4.8 x 40 cm, 700 ml) of IR-45(OH^-)

weakly basic anion exchange resin. The column was eluted with H_2O and the eluate from 350 to 1250 ml was collected and evaporated. Coevaporation of the residue with EtOH and then with acetone gave 11.80 g (54%) of unreacted 72 as a syrup.

The combined organic phase from the above extraction procedure was dried over Na_2SO_4, filtered and evaporated. The residue was coevaporated with toluene (4 x 100 ml) and then with Et_2O (2 x 100 ml). The residue was then dissolved in Et_2O, the resulting suspension was filtered and the filtrate was evaporated to yield 13.77 g (91% based on the amount of 72 recovered) of crude 75 as a pale yellow syrup. A singlet at δ 3.30 in the nmr spectrum of crude 75 was assigned to the OCH_3 resonance of dipivalylated material and indicated <10% of dipivalylation.

5-O-Pivalyl-1,2-O-isopropylidene-β-D-arabinofuranose (76).

A solution of 17.78 g (71.7 mmoles) of 74 in 400 ml of dry, alcohol free acetone containing 3.0 g (8.2 mmoles) of dry HCl was stirred magnetically while protected from moisture by a Drierite drying tube. After 46 hr 9.0 g of $BaCO_3$ was added and stirring was continued overnight. The neutralized solution was filtered through Celite and the filtrate was evapo-

rated to yield a dark yellow syrup which was coevaporated with CH_2Cl_2. The resulting syrup was dissolved in 10 ml of CH_2Cl_2—MeOH (97:3) and applied to a column of 700 g of silica gel (5.4 x 68 cm) packed in the same solvent. The column was eluted successively with CH_2Cl_2—MeOH (97:3, 1.3 ℓ), CH_2Cl_2—MeOH (96:4, 1 ℓ), CH_2Cl_2—MeOH (95:5, 1 ℓ), CH_2Cl_2—MeOH (93:7, 1 ℓ) and CH_2Cl_2—MeOH (91:9, 1 ℓ) at a flow rate of 10-20 ml/min. The eluate from 1825 to 2625 ml was collected, evaporated and coevaporated four times with Et_2O to yield 9.06 g (46%) of 76 as a pale yellow syrup. This material (tlc, $CHCl_3$—MeOH, 95:5, R_f ~ 0.35) contained a trace amount of an impurity material (R_f ~ 0.45). Crystallization from Et_2O—pentane gave 6.98 g (35.5%) of pure 76 as long needles, mp 56-58°C. The column eluate from 1700 to 1825 ml gave a further 2.38 g (12%) of 76 which contained a greater amount of the R_f 0.45 impurity. Crystallization gave 1.13 g (6%) of pure 76, mp 55-57°C (total yield of crystalline 76 is 41.5%). $[\alpha]_D^{21}$ +16.0° (*c* 1, $CHCl_3$); nmr (DMSO-d_6) δ 1.18 (s, 9, $C(CH_3)_3$), 1.27, 1.46 (s, s; 3, 3; $C(CH_3)_2$), 3.95-4.25 (m, 4, H_3, H_4, H_5, $H_{5'}$), 4.47 (d, J_{2-1} = 3.9 Hz, 1, H_2) 5.50 (d, J_{OH-3} ~ 3.5 Hz, 1, 3-*OH*), 5.84 (d, J_{1-2} = 3.9 Hz, 1, H_1); ms m/e 274 (0.36, *M*), 259 (58, *M*-CH_3), 85 (100, $(CH_3)_3C.CO^+$), 57 (290, $(CH_3)C^+$).

Anal. Calcd for $C_{13}H_{22}O_6$: C, 56.90; H, 8.08. Found: C, 56.87; H, 7.89.

1,2-O-Isopropylidene-β-D-arabinofuranose (77).

(a) From 5-O-Pivalyl-1,2-O-isopropylidene-β-D-arabinofuranose (76): To a solution of 3.0 g (130 mmoles) of sodium in 450 ml of dry MeOH was added 15.0 g (54.8 mmoles) of 76. The resulting solution was stirred magnetically while protected from moisture by a Drierite drying tube. After 6.5 hr tlc ($CHCl_3$—MeOH, 9:1) showed quantitative conversion of 76 (R_f ~ 0.5) to 77 (R_f ~ 0.15) and 80 ml of Rexyne 101 strongly acid cation exchange resin, in the pyridinium form, was added. Stirring was continued overnight and the neutralized solution was filtered. The filtrate was evaporated and the residue was coevaporated with toluene—MeOH (2 x 100 ml) and acetone (3 x 100 ml) to yield 10.11 g (97.5%) of crude 77 as a pale yellow solid. This material was dissolved in 50 ml of boiling acetone and 30 ml of Et_2O was added. The resulting cloudy solution was filtered through Celite and the filtrate was evaporated. Crystallization of the residue from acetone—Et_2O—pentane gave 9.46 g (91%) of 77 as colourless rectangular plates: mp 118-120°C; $[\alpha]_D^{22}$ +18.5° (c 1, MeOH); nmr (D_2O, TMS external)

δ 1.57, 1.76 (s, s; 3, 3; C(CH$_3$)$_2$), 3.92 (d, $J_{5,5'-4}$ ~ 7.0 Hz, 2, H_5, $H_{5'}$), 4.2-4.45 (m, 2, H_3, H_4), 4.89 (d, J_{2-1} ~ 3.8 Hz, 1, H_2), 6.21 (d, J_{1-2} ~ 3.8 Hz, 1, H_1).

Anal. Calcd for $C_8H_{14}O_5$: C, 50.52; H, 7.42. Found: C, 50.67; H, 7.36.

(b) From 5-O-p-Toluenesulphonyl-1,2-O-isopropylidene-β-D-arabinofuranose (75): A solution of 0.86 g (2.50 mmoles) of 75 in 250 ml of MeOH containing 0.135 g (2.50 mmoles) of NaOMe was irradiated (quartz vessel, 450 watt medium-pressure Hg lamp) for 1 hr at ~20°C. Tlc (CHCl$_3$—MeOH, 93:7) showed almost complete conversion of 75 (R_f ~ 0.65) to 77 (R_f ~ 0.2) plus minor impurity products (R_f's ~ 0.5 and 0.8). The reaction mixture was evaporated, the brown residue was dissolved in MeOH—H$_2$O (1:1, 40 ml) and this solution was extracted with Et$_2$O—pentane (1:1, 4 x 20 ml) to effect removal of the R_f 0.8 impurity. The organic phase was discarded and the aqueous phase was diluted with 40 ml of H$_2$O, salted with NaCl and extracted with EtOAc (10 x 30 ml). The combined organic phase was dried over Na$_2$SO$_4$, filtered and evaporated to yield 0.356 g (75%) of crude 77 as a yellow syrup. Crystallization from CHCl$_3$—skellysolve B gave (2 crops) 0.187 g (36%) of 77 as yellow granules: mp 116-119°C.

3,5-Di-O-benzyl-1,2-O-isopropylidene-β-D-arabinofuranose (78).

In a 500 ml round bottomed flask, protected by a Drierite drying tube, 7.68 g (160 mmoles, as a 50% dispersion in oil) of NaH was washed by decantation with pentane (3 x 50 ml). Excess pentane was removed by gentle warming and 150 ml of dry DMF was added. The resulting magnetically stirred suspension was cooled to -10°C and a solution of 7.60 g (40.0 mmoles) of 77 in 75 ml of dry DMF was added dropwise (0.5 hr). Stirring was continued for a further 1.5 hr at 0°C until the gentle effervescence ceased. To the resulting suspension of the disodium salt of 77 was added dropwise (1 hr) a solution of 15.05 g (88.0 mmoles) of benzyl bromide in 75 ml of dry DMF and stirring was continued for a further 0.5 hr at 0°C. The reaction mixture was then poured into 1 ℓ of ice water and the resulting emulsion was vigorously stirred for 0.5 hr. This mixture was extracted with Et_2O (3 x 250 ml) and the combined organic phase was washed with H_2O (4 x 100 ml) and saturated NaCl solution (2 x 100 ml). The organic phase was dried over $CaCl_2$, filtered and the filtrate evaporated. The residue was coevaporated with toluene (100 ml), acetone (100 ml) and Et_2O (3 x 100 ml). The residual syrup was then dissolved in Et_2O and the

resulting suspension (NaBr) was filtered through Celite. The filtrate was evaporated to yield 14.74 g (99%) of chromatographically homogenous (tlc, $CHCl_3$, R_f ~ 0.4) 78 as a pale yellow syrup: $[\alpha]_D^{23}$ +20.0° c 1.98, MeOH); nmr ($CDCl_3$) δ 1.32, 1.44 (s, s; 3, 3; $C(CH_3)_2$), 3.63 (d, $J_{5,5'-4}$ = 6.2 Hz, 2, H_5, $H_{5'}$), 4.03 (d, J_{3-4} = 3.0 Hz, 1, H_3), 4.27 (t of d, J_{4-3} = 3.0 Hz, $J_{4-5,5'}$ = 6.2 Hz, 1, H_4), 4.54, 4.56 (s, s; 2, 2; 3+5-$OCH_2C_6H_5$), 4.62 (d, J_{2-1} = 4.0 Hz, 1, H_2), 5.88 (d, J_{1-2} = 4.0 Hz, 1, H_1), 7.30 (s, 10, 3+5-$OCH_2C_6H_5$); ms m/e 370.1768 (18, M, calcd for $C_{22}H_{26}O_5$: 370.1780), 355 (22, M-CH_3), 312 (51, M-CH_3COCH_3), 279 (76, M-C_7H_7), 203 (100), 91 (3,700, $C_7H_7^+$).

3,5-Di-O-benzyl-D-arabinose (79).

To 14.50 g (39.2 mmoles) of 78 was added 200 ml of 50% acetic acid and 4.0 ml of concentrated hydrochloric acid. The resulting mixture was stirred magnetically and heated to 80°C. After 0.5 hr an homogenous solution had resulted and tlc ($CHCl_3$—MeOH, 98:2) showed complete conversion of 78 (R_f ~ 0.5) to 79 (R_f ~ 0.2). The reaction mixture was evaporated to low volume (~30 ml) and the resulting solution was coevaporated with toluene (4 x 150 ml), each time to low volume. The residual solution was partitioned between $CHCl_3$ (200 ml) and H_2O (100 ml) and the aqueous

phase was extracted with a further 100 ml of $CHCl_3$. The combined organic phase was washed with saturated $NaHCO_3$ solution (50 ml), H_2O (3 x 50 ml) and saturated NaCl (50 ml), and was then dried over Na_2SO_4. The drying agent was filtered and the filtrate was evaporated and then coevaporated with Et_2O (100 ml). The residual syrup was treated with Et_2O (100 ml) and a gelatinous precipitate was filtered using Celite. The filtrate was evaporated and the residue was coevaporated with Et_2O (2 x 100 ml) to yield 12.52 g (97%) of chromatographically homogenous 79 as a pale yellow syrup: $[\alpha]_D^{22}$ +37.1° (c 1.3, MeOH); nmr (DMSO-d_6) δ 4.95, 5.35 (d, d, J_{OH-2} ~ 6.5, 5.0 Hz, 1, α,β 2-OH), 6.22, 6.30 (d, d, J_{OH-1} ~ 4.5, 6.5 Hz, 1, α,β 1-OH), 7.31 (s, 10, 3+5-$OCH_2C_6H_5$), (DMSO-d_6—D_2O) δ 5.07, 5.10 (d, d, J_{1-2} ~ 2.0, 4.0 Hz, 1, α,β-H_1).

A small sample (~0.03 g) of 79 was dissolved in 0.3 ml of dry benzene and 0.3 ml of hexamethyldisilazane. After stirring for 3 days, while protected from moisture by a Drierite drying tube, the reaction mixture was evaporated and then coevaporated with benzene. The residue was dissolved in benzene and filtered through Celite. The filtrate was evaporated to give a crude syrup of 3,5-di-O-benzyl-1,2-bis-O-trimethylsilyl-D-arabinofuranose (80): ms m/e 474.2271 (1.1, M, calcd for $C_{25}H_{38}O_5Si_2$: 474.2257),

235 (100), 91 (490, $C_7H_7^+$).

3,5-Di-O-benzyl-D-arabinofuranosyl Chloride (86).

A solution of 1.69 g (5.13 mmoles) of 79 in 15 ml of dry CH_2Cl_2 containing 2.0 g of Drierite was cooled to 0°C. Dry HCl was bubbled gently through the mixture and conversion of 79 to 86 was assayed by tlc as follows. Aliquots of the reaction mixture were added to a suspension of barium carbonate in methanol. Neutralization of the acid was rapid and 86 present in the reaction mixture was quantitatively converted to methyl 3,5-di-O-benzyl-D-arabinofuranose (89). After 15 min, tlc ($CHCl_3$—MeOAc, 8:2) showed complete conversion of 79 ($R_f \sim 0.15$) to 89 ($R_f \sim 0.4$). A small amount of crude, syrupy 89 was obtained by filtration and evaporation: nmr ($CDCl_3$) δ 3.39 (s, 3, α,β-OCH_3), 4.82 (d, J_{1-2} = 4.5 Hz, β-H_1), 4.87 (s, α-H_1), 7.28 (s, 10, 3+5-$OCH_2C_6H_5$). Filtration of the chlorination reaction after 30 min gave a CH_2Cl_2 solution of 86 which produced an homogenous spot (tlc) for 89 after treatment as described. Solutions of 86 prepared in this manner were used immediately in subsequent condensation reactions as described.

When an aliquot of the solution of 86 was evaporated to dryness and the residual oil was successively coevaporated to dryness four times with toluene,

extensive decomposition of 86 was noted. Tlc of a MeOH solution of the resulting syrup showed a small spot for 89 at R_f ~ 0.4 which indicated that a minor amount (~20%) of 86 remained in the syrup. A major spot at R_f ~ 0.5 was presumed to arise by self condensation of molecules of 86 to give di or polysaccharide by-products (90) (see Results and Discussion section).

Phenyl 3,5-di-O-benzyl-1-thio-α-D-arabinofuranoside (87).

To a suspension of 0.36 g (15.0 mmoles) of NaH in 20 ml of dry THF stirred magnetically under dry nitrogen was added dropwise a solution of 1.53 ml (1.65 g, 15.0 mmoles) of benzenethiol in 10 ml of dry THF. Vigorous effervescence occurred and a colourless precipitate of sodium phenylmercaptide separated. This suspension was cooled to 0°C in an ice bath. A solution of 86 in CH_2Cl_2 (derived from 7.6 mmoles of 79) was evaporated to dryness and the residue was coevaporated to dryness five times with 50 ml portions of dry benzene. The residue was dissolved in 20 ml of dry THF and the resulting solution was added dropwise (45 min) to the vigorously stirred suspension of sodium phenylmercaptide. After stirring a further 3 hr at 0°C and 22 hr at room temperature, the reaction

mixture was poured into 50 ml of saturated $NaHCO_3$ solution. Et_2O (20 ml) was added, the organic phase was separated and the aqueous phase was extracted with a further portion (20 ml) of Et_2O. The combined organic phase was washed with saturated $NaHCO_3$ solution (2 x 25 ml) and H_2O (2 x 25 ml) and was then dried over $MgSO_4$. The drying agent was filtered and the filtrate evaporated to yield crude 87 as a yellow syrup (3.3 g) (tlc, $CHCl_3$ developed twice, R_f ~ 0.55) containing large amounts of slower migrating by-products (R_f's ~ 0.4, 0.2 and 0.1). This syrup was dissolved in a minimal volume of $CHCl_3$ and applied to a column (4.8 x 37 cm, 200 g) of silica gel packed in $CHCl_3$. The column was eluted with $CHCl_3$ and the eluate from 900 to 1175 ml was collected and evaporated. Coevaporation of the residue with acetone (2 x 50 ml) gave 0.75 g (24%) of chromatographically homogenous 87 as a colourless syrup: $[\alpha]_D^{23}$ +189.5° (*c* 0.84, 95% EtOH); nmr ($CDCl_3$) δ 3.53, 3.70 (ABq of doublets, $J_{5-5'}$ = 10.3 Hz, $J_{5,5'-4}$ = 2.4 Hz, 2, H_5, $H_{5'}$), 3.94 (m, 1, H_3), 4.3-4.75 (multiplets, 6, H_2, H_4, 3+5-$OCH_2C_6H_5$), 5.50 (poorly resolved t, J ~ 0.65 Hz, 1, H_1), 7.15-7.55 (m, 15, 3+5-$OCH_2C_6H_5$, 1-SC_6H_5); uv (MeOH) 248.5 nm (ε 7,800); ms m/e 422 (7.2, *M*), 313 (55, *M*-C_6H_5S), 205 (100, 313-C_7H_7OH), 91 (~1200, $C_7H_7^+$).

Phenyl 3,5-di-*O*-benzyl-1-thio-β-*D*-arabinofuranoside (**88**).

The title compound was prepared in an analogous procedure to that used above in the preparation of **87** except that the condensation reaction was carried out in the presence of a 2 fold excess of benzenethiol. Thus, a suspension of sodium phenylmercaptide in THF was prepared by treatment of 0.24 g (10.0 mmoles) of NaH with 2.04 ml (2.20 g, 20.0 mmoles) of benzenethiol. Treatment of the suspension with a THF solution of **86** derived from 5.0 mmoles of **79** gave 0.40 g (19%) of **88** as a colourless syrup after column chromatography. Tlc (CH_2Cl_2) showed a single spot for **88** ($R_f \sim 0.4$) which migrated faster than **87** ($R_f \sim 0.3$). $[\alpha]_D^{23}$ -118.5° (*c* 1.04, MeOH); nmr ($CDCl_3$—D_2O) δ 3.56 (q, $J_{5-5'}$ = 10.5 Hz, J_{5-4} = 2.7 Hz, 1, H_5), 3.85 (q, $J_{5-5'}$ = 10.5 Hz, J_{5-4} = 2.5 Hz, 1, $H_{5'}$), 3.99 (d, J_{3-2} = 1.4 Hz, 1, H_3), 4.19 (t of d, $J_{4-5,5'} \sim 2.5$ Hz, $J_{4-3} \sim 1.4$ Hz, 1, H_4), 4.26 (br d, $J_{2-1} \sim 3.0$ Hz, 1, H_2) 4.46, 4.61 (ABq, J_{GEM} = 11.8 Hz, 2, 3 or 5-$OCH_2C_6H_5$), 4.47, 4.69 (ABq, J_{GEM} = 11.9 Hz, 2, 3 or 5-$OCH_2C_6H_5$), 5.48 (d, J_{1-2} = 3.0 Hz, 1, H_1), 7.1-7.6 (m, 15, 3+5-$OCH_2C_6H_5$ and 1-SC_6H_5); ms m/e 422.1562 (18, *M*, calcd for $C_{25}H_{26}O_4S$: 422.1552), 313 (97, *M*-C_6H_5S), 205 (100, 313-C_7H_7OH), 91 (220, $C_7H_7^+$).

Benzyl 3,5-di-O-benzyl-1-thio-α-D-arabinofuranoside (92).

To a suspension of 0.18 g (7.5 mmoles) of NaH in 30 ml of dry THF, stirred magnetically under dry nitrogen at 0°C, was added dropwise a solution of 0.81 ml (0.86 g, 6.8 mmoles) of α-toluenethiol in 20 ml of dry THF. Vigorous effervescence occurred and a colourless precipitate of sodium benzylmercaptide was formed. A solution of 86 in CH_2Cl_2 (derived from 3.42 mmoles of 79) was diluted with toluene (100 ml) and the resulting solution evaporated to low volume (~10 ml). This solution was diluted with dry THF (100 ml) and again evaporated to low volume. Dilution to ~40 ml with dry THF gave a solution of 86 which showed (tlc) little conversion to polysaccharide by-products. This solution was added dropwise (15 min) to the stirred suspension of sodium benzylmercaptide and stirring was continued for a further 2 hr at 0°C. Et_2O (75 ml) was added to the reaction mixture and the resulting solution was washed with H_2O (3 x 50 ml). The organic phase was evaporated and the residue coevaporated successively with EtOH, $CHCl_3$ and CH_2Cl_2. The resulting dark yellow syrup of crude 92 was dissolved in a minimal volume of CH_2Cl_2 and applied to a column (2.9 x 50 cm, 130 g) of silica gel packed in CH_2Cl_2.

The column was eluted with CH_2Cl_2 and the eluate from 1150 to 1750 ml was collected and evaporated. Coevaporation of the residue with Et_2O (2 x 50 ml) gave 1.04 g (70%) of chromatographically homogenous (tlc, CH_2Cl_2, $R_f \sim 0.25$) *92* as a pale yellow syrup: $[\alpha]_D^{22}$ +240° (*c* 1.2, MeOH); nmr (DMSO-d_6) δ 3.61 (d, $J_{5,5'-4} \sim 4.5$ Hz, 2, H_5, $H_{5'}$), 3.74 (m, 1, H_3), 3.80 (s, 2, 1-S$CH_2$$C_6H_5$), 4.03 (m, 1, H_2), 4.19 (br q, 1, H_4), 4.52 (s, 2, 3 or 5-O$CH_2$$C_6H_5$), 4.48, 4.65 (ABq, $J_{GEM} = 12.0$ Hz, 2, 3 or 5-O$CH_2$$C_6H_5$), 4.95 (d, $J_{1-2} = 2.7$ Hz, 1, H_1), 5.70 (d, $J_{OH-2} = 4.8$ Hz, 1, 2-*OH*), 7.31 (s, 15, 3+5-$OCH_2C_6H_5$ and 1-$SCH_2$$C_6H_5$), (DMSO-$d_6$—$D_2O$) δ 4.03 (t, $J_{2-1} \sim J_{2-3} \sim 2.7$ Hz, 1, H_2), doublet at 5.70 disappears; ms m/e 436.1702 (5.2, *M*, calcd for $C_{26}H_{28}O_4S$: 436.1709), 313 (100, *M*-C_7H_7S), 312 (97, *M*-C_7H_7SH), 253 (77, *M*-$C_{14}H_{15}$), 205 (100, 313-C_7H_7OH), 91 (~2,700, $C_7H_7^+$). In a subsequent experiment a middle fraction from the column chromatography was evaporated and dried *in vacuo* to an analytically pure sample of syrupy *92*: $[\alpha]_D^{22}$ +265° (*c* 1.48, MeOH).

Anal. Calcd for $C_{26}H_{28}O_4S$: *C*, 71.53; *H*, 6.46; *S*, 7.35. Found: *C*, 71.64; *H*, 6.66; *S*, 7.37.

Benzyl 2-*O*-Methanesulphonyl-3,5-di-*O*-benzyl-1-thio-α-*D*-arabinofuranoside (*94*).

To a solution of 1.31 g (3.0 mmole) of *92* in

10 ml of dry pyridine, stirred magnetically at 0°C and protected from moisture by a Drierite drying tube, was added 0.70 ml (1.03 g, 9.0 mmole) of methanesulphonyl chloride. After stirring 2 hr at 0°C, tlc (CH_2Cl_2) showed complete conversion of 92 (R_f ~ 0.2) to 94 (R_f ~ 0.4). H_2O (0.5 ml) was added, stirring was continued for 10 min and the reaction mixture was then diluted with ether (50 ml) and ice cold H_2O (25 ml). The aqueous phase was separated and the organic phase was washed successively with ice cold solutions of saturated $NaHCO_3$ (25 ml), H_2O (2 x 25 ml) and saturated NaCl (2 x 25 ml). The organic phase was dried over Na_2SO_4, the drying agent was filtered and the filtrate was evaporated. The colourless syrupy residue was coevaporated successively with toluene (50 ml) and ether (3 x 50 ml) to yield 1.56 g (quantitative) of 94. Tlc of this product showed, besides the major spot for 94, a very faint spot at R_f ~ 0.05. This material was used immediately without further purification in subsequent reactions. This sample had $[\alpha]_D^{23}$ +213° (c 1.4, $CHCl_3$); nmr ($CDCl_3$) δ 2.74 (s, 3, CH_3SO_3), 3.62 (m, 2, H_5, $H_{5'}$), 3.75, 3.91 (ABq, J_{GEM} = 13.7 Hz, 2, 1-$SCH_2C_6H_5$), 4.15 (m, J_{3-4} = 6.5 Hz, 1, H_3), 4.34 (m, J_{4-3} = 6.5 Hz, 1, H_4), 4.44, 4.58 (ABq, J_{GEM} = 12.0 Hz, 2, 3 or 5-$OCH_2C_6H_5$), 4.51, 4.72 (ABq, J_{GEM} = 11.7 Hz,

2, 3 or 5-$OCH_2C_6H_5$), 5.00 ("t", J_{2-1} = 1.9 Hz, J_{2-3} ~ 2.2 Hz, 1, H_2), 5.22 (d, J_{1-2} = 1.9 Hz, 1, H_1), 7.30 (br s, 15, 3+5-$OCH_2C_6H_5$ and 1-$SCH_2C_6H_5$); ir 1182, 1365 cm^{-1} (CH_3SO_3); ms m/e 310.1033 (14, *M*-CH_3SO_3H-C_7H_7OH, calcd for $C_{19}H_{18}O_2S$: 310.1028), 91 (100, $C_7H_7^+$).

Phenyl 2-*O*-methanesulphonyl-3,5-di-*O*-benzyl-1-thio-α-*D*-arabinofuranoside (93).

Conversion of 87 to 93 proceeded analogously to the conversion of 92 to 94 described above. The reaction of 0.125 g (0.296 mmoles) of 87 with 0.222 g (1.94 mmoles) of methanesulphonyl chloride in 2 ml of dry pyridine gave 0.146 g (98%) of chromatographic homogenous (tlc, $CHCl_3$, R_f ~ 0.35) 93 as a colourless syrup: nmr ($CDCl_3$) δ 2.91 (s, 3, CH_3SO_3), 3.62 (d, $J_{5,5'-4}$ ~ 3.7 Hz, 2, H_5, $H_{5'}$), 4.2-4.7 (multiplets, 6, H_3, H_4, 3+5-$OCH_2C_6H_5$), 5.14 (t, J_{2-1} ~ J_{2-3} ~ 2.5 Hz, 1, H_2), 5.62 (d, J_{1-2} ~ 2.5 Hz, 1, H_1), 7.15-7.6 (m, 15, 3+5-$OCH_2C_6H_5$ and 1-SC_6H_5).

Reaction of 93 with *n*-propanol.

To a solution of 58 mg (0.115 mmoles) of 93 in 1 ml of dry DMF was added 0.25 ml (3.3 mmoles) of *n*-propanol. The resulting solution was stirred magnetically while protected from moisture by a Drierite

drying tube and heated at 55-60°C. After 65 hr tlc pentane—ether, 7:3) showed good conversion of **93** (R_f ~ 0.23) to a faster migrating product (R_f ~ 0.55). The reaction mixture was diluted with $CHCl_3$ (10 ml) and washed with saturated $NaHCO_3$ solution (10 ml) and H_2O (2 x 10 ml). The organic phase was dried over Na_2SO_4, filtered and evaporated. The mass spectrum of the resulting syrup was compatible with the formation of *n*-propyl 2-*S*-phenyl-3,5-di-*O*-benzyl-2-thio-β and/or α-D-ribofuranoside (**96** and/or **97**): m/e 464 (8.0, *M*), 404 (9.5, *M*-C_3H_7OH), 267 (34, 404-C_6H_5S-CO), 255 (100, 404-C_7H_7O-CH_2CO), 91 (~540, $C_7H_7^+$). Preparative tlc (pentane—ether, 9:1, developed twice) of the syrup resolved three major components. A band at R_f 0.27 yielded 23 mg of a syrup tentatively identified as the β-anomer (**96**): nmr ($CDCl_3$) δ 0.82 (t, J = 7.0 Hz, 3, $CH_2\underline{CH_3}$), 1.47 (sextet, J ~ 7 Hz, 2, $CH_2\underline{CH_2}CH_3$), 3.25, 3.59 (ABq of triplets, J_{GEM} = 9.5 Hz, J_{vic} ~ 6.5 Hz, 2, $O\underline{CH_2}CH_2$), 3.56 (d, $J_{5,5'-4}$ = 3.8 Hz, 2, H_5, $H_{5'}$), 6.10 (q, J_{2-1} = 2.3 Hz, J_{2-3} = 5.5 Hz, 1, H_2), 4.24-4.40 (multiplets, 2, H_3, H_4), 4.55 (s, 2, 3 or 5-$O\underline{CH_2}C_6H_5$), 4.47, 4.69 (ABq, J_{GEM} = 11.5 Hz, 2, 3 or 5-$O\underline{CH_2}C_6H_5$), 5.05 (d, J_{1-2} = 2.3 Hz, 1, H_1), 7.32 (m, 15, 3+5-$OCH_2\underline{C_6H_5}$ and 2-SC_6H_5).

A band at R_f ~ 0.14 on the preparative tlc plate gave 15 mg of a syrup which was tentatively identified

as the α-anomer (97): nmr ($CDCl_3$) δ 0.92 (t, J = 7.3 Hz, 3, CH_2CH_3), 1.64 (sextet, J ~ 7 Hz, 2, $CH_2CH_2CH_3$), 3.2-3.8 (multiplets, 5, H_2, H_5, H_5', OCH_2CH_2), 4.0 (d of d, J_{3-2} = 7.0 Hz, J_{3-4} = 2.7 Hz, 1, H_3), 4.27 (br t of d, J_{4-3} ~ 2.7 Hz, $J_{4-5,5'}$ ~ 4.0 Hz, 1, H_4), 4.44 (s, 2, 3 or 5-$OCH_2C_6H_5$), 4.49, 4.69 (ABq, J_{GEM} = 12.5 Hz, 2, 3 or 5-$OCH_2C_6H_5$), 5.14 (d, J_{1-2} = 4.75 Hz, 1, H_1), 7.25 (m, 15, 3+5-$OCH_2C_6H_5$ and 2-SC_6H_5).

A third band at R_f 0.36 gave 6 mg of a syrup which was subsequently identified as the 2-phenylthioglycal (98) (see reaction of 93 with ethanol).

Reaction of 93 with ethanol in the presence of barium carbonate.

To a solution of 29 mg (0.058 mmoles) of 93 in 1 ml of dry CH_3CN containing 0.03 ml (0.515 mmoles) of EtOH was added 45 mg (0.23 mmoles) of barium carbonate. The mixture was stirred magnetically while protected from moisture by a Drierite drying tube and heated at 75-80° for 20 hr. Tlc (pentane—ether, 7:3) showed good conversion of 93 (R_f ~ 0.22) to a faster migrating product (R_f ~ 0.5). The reaction mixture was diluted with $CHCl_3$ (10 ml) and filtered through Celite. The filtrate was washed with saturated $NaHCO_3$ solution (5 ml) and H_2O (3 x 5 ml). The organic phase was dried over Na_2SO_4, filtered and the filtrate

evaporated. Preparative tlc of the resulting syrup (pentane—ether, 8:2) revealed two major bands at R_f's ~ 0.51 and 0.61. The R_f 0.51 band yielded 15 mg of ethyl 2-*S*-phenyl-3,5-di-*O*-benzyl-2-thio-2-deoxy-β-D-ribofuranoside (99) as a colourless syrup: nmr ($CDCl_3$) δ 1.08 (t, J = 6.9 Hz, 3, $CH_2\underline{CH_3}$), 3.36, 3.69 (ABq of quartets, J_{GEM} = 9.8 Hz, J_{vic} = 6.9 Hz, 2, $O\underline{CH_2}CH_3$), 3.56 (m, 2, H_5, $H_{5'}$), 3.88 (d of d, J_{2-1} ~ 2.1 Hz, J_{2-3} ~ 5.5 Hz, 1, H_2), 4.33 (m, 2, H_3, H_4), 4.55 (s, 2, 3 or 5-$O\underline{CH_2}C_6H_5$), 4.47, 4.70 (ABq, J_{GEM} = 11.3 Hz, 2, 3 or 5-$O\underline{CH_2}C_6H_5$), 5.06 (d, J_{1-2} = 2.1 Hz, 1, H_1), 7.30 (m, 15, 3+5-$OCH_2\underline{C_6H_5}$ and 2-$S\underline{C_6H_5}$); ms m/e 450 (13, M), 404 (8.6, M-C_2H_5OH), 267 (43, 404-C_6H_5S-CO), 255 (100, 404-C_7H_7O-CH_2CO), 91 (~1020, $C_7H_7^+$).

The R_f 0.61 band gave 6 mg of a colourless syrup which was tentatively identified as 2-*S*-phenyl-3,5-di-*O*-benzyl-2-thio-D-arabinal (98): nmr ($CDCl_3$) δ 3.54 (d, $J_{5,5'-4}$ = 4.8 Hz, 2, H_5, $H_{5'}$), 4.54 (s overlapping m, 5, 3+5-$O\underline{CH_2}C_6H_5$ and H_3), 4.69 (m, 1, H_4), 6.88 (s, 1, H_1), 7.1-7.4 (m, 15, 3+5-$OCH_2\underline{C_6H_5}$ and 2-$S\underline{C_6H_5}$); ms m/e 404 (100, M), 296 (33, M-C_7H_7OH), 283 (20, M-C_7H_7-CH_2O), 267 (13, M-C_6H_5S-CO), 91 (~470, $C_7H_7^+$).

Methyl 2-S-benzyl-3,5-di-O-benzyl-2-thio-2-deoxy-β-D-ribofuranoside (100).

To a solution of 2.06 g (4.0 mmoles) of 94 in 30 ml of dry CH_3CN was added 1.28 g (40 mmoles) of MeOH and 2.36 g (12.0 mmoles) of $BaCO_3$. The mixture was stirred magnetically while protected from moisture by a Drierite drying tube and heated at 60°C. After 12.5 hr the reaction mixture was allowed to cool, diluted with 50 ml of ether, filtered through Celite and the filtrate was evaporated. The residual yellow syrup was dissolved in a minimal volume of $CHCl_3$ and applied to 4 preparative tlc plates (20 x 40 cm) which were developed in pentane—ether (7:3). The major band (R_f ~ 0.5) was eluted with $CHCl_3$—MeOH (9:1) and the eluate was evaporated. The residue was co-evaporated with ether (3 x 30 ml) to yield 1.45 g (80.5%) of chromatographically homogenous 100 as a colourless syrup: $[\alpha]_D^{23}$ +57.0° (c 1.98, MeOH); nmr ($CDCl_3$) δ 3.22 (d of d, J_{2-1} ~ 2.8 Hz, J_{2-3} = 6.1 Hz, 1, H_2), 3.30 (s, 3, OCH_3), 3.46 (d, $J_{5,5'-4}$ = 5.2 Hz, 2, H_5, H_5'), 3.78 (s, 2, 2-$SCH_2C_6H_5$), 4.06 (d of d, J_{3-2} ~ 6.0 Hz, J_{3-4} ~ 5.0 Hz, 1, H_3), 4.23 ("q" $J_{4-5,5'}$ ~ J_{4-3} ~ 5.0 Hz, 1, H_4), 4.35, 4.57 (ABq, J_{GEM} = 11.7 Hz, 2, 3 or 5-$OCH_2C_6H_5$), 4.49 (s, 2, 3 or 5-$OCH_2C_6H_5$), 4.96 (d, J_{1-2} = 2.8 Hz, 1, H_1), 7.26 (br s, 15, 3+5-$OCH_2C_6H_5$ and 2-$SCH_2C_6H_5$); ms m/e

450.1850 (0.55, M, calcd for $C_{27}H_{30}O_4{}^{32}S$: 450.1865), 418 (18, M-CH_3OH), 327 (8.1, 418-C_7H_7), 299 (18, 327-CO), 295 (9.7, 418-C_7H_7S), 269 (100, 418-C_7H_7O-CH_2CO), 91 (~1100, $C_7H_7{}^+$).

Ethyl 2-S-benzyl-3,5-di-O-benzyl-2-thio-2-deoxy-β-D-ribofuranoside (101).

A solution of 0.49 g (0.95 mmoles) of 94 in 5 ml of dry CH_3CN was treated with 0.46 g (10.0 mmoles) of EtOH and 0.59 g (3.0 mmoles) of $BaCO_3$ as described above in the preparation of 100. The major band from the preparative tlc plates (R_f ~ 0.45) yielded 0.322 g (73%) of syrupy 101: $[\alpha]_D^{23}$ +41.5° (c 1.3, $CHCl_3$); nmr ($CDCl_3$) δ 1.14 (t, J = 7.0 Hz, 3, $CH_2\underline{CH_3}$), 3.21 (d of d, J_{2-1} = 3.2 Hz, J_{2-3} = 6.0 Hz, 1, H_2), 3.46 (d, $J_{5,5'-4}$ = 5.2 Hz, 2, H_5, $H_{5'}$), 3.44, 3.75 (ABq of quartets, J_{GEM} = 10.0 Hz, J_{vic} = 7.0 Hz, 2, $O\underline{CH_2}CH_3$), 3.78 (s, 2, 2-$S\underline{CH_2}C_6H_5$), 4.06 ("q", J_{3-4} ~ 4.7 Hz, J_{3-2} ~ 6.0 Hz, 1, H_3) 4.21 ("q", J_{4-3} ~ $J_{4-5,5'}$ ~ 5 Hz, 1, H_4), 4.36, 4.54 (ABq, J_{GEM} = 11.5 Hz, 2, 3 or 5-$O\underline{CH_2}C_6H_5$), 4.48 (s, 2, 3 or 5-$O\underline{CH_2}C_6H_5$), 5.08 (d, J_{1-2} = 3.2 Hz, 1 H_1), 7.26 (br s, 15, 3+5-$OCH_2\underline{C_6H_5}$ and 2-$SCH_2\underline{C_6H_5}$); ms m/e 464.2037 (0.18, M, calcd for $C_{28}H_{32}O_4{}^{32}S$: 464.2022), 418 (9.5, M-C_2H_5OH), 327 (5.2, 418-C_7H_7), 310 (15, 418-C_7H_7OH), 299 (23, 327-CO), 269 (100, 418-C_7H_7O-CH_2CO), 91 (~780, $C_7H_7{}^+$).

A minor band at R_f ~ 0.57 yielded 4 mg (~1%) of a syrupy product that was subsequently identified as the 2-benzylthioglycol (103) (see reaction of 94 with 6-chloropurine).

iso-Propyl 2-S-benzyl-3,5-di-O-benzyl-2-thio-2-deoxy-β-D-ribofuranoside (102).

A solution of 1.04 g (2.0 mmoles) of 94 in 15 ml of dry CH_3CN was treated with 1.20 g (20.0 mmoles) of iso-propanol and 1.18 g (6.0 mmole) of $BaCO_3$ as described above in the preparation of 100. Preparative tlc of the resulting crude syrup of 102 on three plates (20 x 40 cm) developed three times in pentane—ether (9:1) gave a narrow separation of 102 (R_f ~ 0.42) and 103 (R_f ~ 0.53). The R_f 0.42 band yielded 0.702 g (73%) of 102 as a colourless syrup: $[\alpha]_D^{23}$ +41.9° (c 1.84, MeOH); nmr ($CDCl_3$) δ 1.15 (d, J = 6.0 Hz, 6, $CH(CH_3)_2$), 3.18 (d of d, J_{2-1} = 3.5 Hz, J_{2-3} = 6.0 Hz, 1, H_2), 3.47 (d, $J_{5,5'-4}$ = 5.5 Hz, 2, H_5, H_5'), 3.89 (septet, J = 6.0 Hz, 1, $OCH(CH_3)_2$), 4.03 (m, J_{3-4} ~ 4.5 Hz, 1, H_3), 4.21 (br "q", J_{4-3} ~ $J_{4-5,5'}$ ~ 5 Hz, 1, H_4), 4.38, 4.54 (ABq, J_{GEM} = 12.0 Hz, 2, 3 or 5-OCH_2-C_6H_5), 4.49 (s, 2, 3 or 5-$OCH_2C_6H_5$), 5.22 (d, J_{1-2} = 3.5 Hz, 1, H_1), 7.28 (m, 15, 3+5-$OCH_2C_6H_5$ and 2-$SCH_2C_6H_5$); ms m/e 478.2147 (0.15, M, calcd for $C_{29}H_{34}O_4{}^{32}S$: 478.2178), 418 (6.8, M-C_3H_7OH), 327

(5.4, 418-C_7H_7), 310 (16, 418-C_7H_7OH), 299 (39, 327-CO), 295 (8.8, 418-C_7H_7S), 269 (100, 418-C_7H_7O-CH_2CO), 91 (~860, $C_7H_7^+$).

Methyl 2-deoxy-β-D-*erythro*-pentofuranoside (**104**).

To a solution of 1.39 g (3.09 mmoles) of **100** in 50 ml of 98% EtOH was added 22.5 g (wet weight, prewashed with 98% EtOH) of Raney Nickel and the mixture was refluxed for 1 hr. The hot reaction mixture was filtered through Celite and the filter pad was washed with boiling MeOH (4 x 30 ml). The combined filtrate was evaporated and the residue was dissolved in acetone. The resulting suspension was filtered through Celite, the filtrate evaporated and the residue was coevaporated with acetone to yield 0.454 g (99%) of **104** as a pale yellow syrup. Tlc of this product ($CHCl_3$-MeOH, 95:5) showed a major spot for **104** (R_f ~ 0.1) which migrated faster than 2-deoxy-D-ribose (R_f ~ 0.04). An extremely faint spot (less than 5%) at R_f ~ 0.4 was presumed to be incompletely debenzylated material. This syrup had $[\alpha]_D^{23}$ -102.2° (*c* 1.65, MeOH); nmr (DMSO-d_6) δ 1.94 (m, 2, H_2, H_2'), 3.20 (s, 3, OCH_3), 3.38 (m, 2, H_5, H_5'), 3.70 (m, 1, H_4), 410 (m, J_{3-4} ~ 4.1 Hz, $J_{3-2,2'}$ ~ 6.0 Hz, 1, H_3), 4.56 (br t, $J_{OH-5,5'}$ ~ 5.5 Hz, 1, 5-OH), 4.94 (br d, 1, 3-OH), 4.96 (d of d, J_{1-2} ~ 4.8 Hz, $J_{1-2'}$ ~ 3.0 Hz, 1, H_1). Reported[222)

for the α-anomer of 104: $[\alpha]_D^{20}$ +70.0° (c 2, H_2O).

Ethyl 2-deoxy-β-D-erythro-pentofuranoside (105).

Desulphurization-debenzylation of 0.29 g (0.625 mmoles) of 101 using 3.26 g of Raney Nickel was effected as described above in the preparation of 104 to yield 0.094 g (93%) of 105 as a pale yellow syrup: $[\alpha]_D^{22}$ -63.7° (c 1.06, MeOH); nmr (DMSO-d_6) δ 1.09 (t, J = 7.0 Hz, 3, CH_2CH_3), 1.96 (m, J_{2-3} = 5.5 Hz, $J_{2'-3}$ = 6.0 Hz, 2, H_2, $H_{2'}$), 3.2-3.8 (multiplets, 5, H_4, H_5, $H_{5'}$, OCH_2CH_3), 4.12 (t of d, J_{3-4} ~ 3.7 Hz, J_{3-2} ~ $J_{3-2'}$ ~ 5.7 Hz, 1, H_3), 4.60 (broad peak, 2, 3-OH, 5-OH), 5.09 (d of d, J_{1-2} = 3.5 Hz, $J_{1-2'}$ = 5.0 Hz, 1, H_1), a small peak at δ 7.32 indicated the presence of ~3% of a presumed benzylated impurity in product 105. Reported[222)] for the α-anomer of 105: $[\alpha]_D^{20}$ +48.8° (c 2, H_2O).

iso-Propyl 2-deoxy-β-D-erythro-pentofuranoside (106).

Desulphurization-debenzylation of 0.702 g (1.47 mmoles) of 102 using 10.4 g of Raney Nickel was effected as described above in the preparation of 104 to yield 0.254 g (100%) of 106 as a colourless syrup: $[\alpha]_D^{23}$ -99.2° (c 1.69, MeOH); nmr (DMSO-d_6) δ 1.06, 1.10 (d, d,

J = 6.3 Hz, 6, $CH(CH_3)_2$), 1.91 (m, $J_{2,2'-1}$ ~ 4.5 Hz, $J_{2,2'-3}$ ~ 5.7 Hz, 2, H_2, $H_{2'}$), 3.38 (m, $J_{5,5'-4}$ ~ 6.5 Hz, 2, H_5, $H_{5'}$), 3.70 (m, 1, H_4), 3.81 (septet, J = 6.3 Hz, 1, $OCH(CH_3)_2$), 4.12 (m, J_{3-4} ~ 3.5 Hz, $J_{3-2,2'}$ ~ 5.7 Hz, 1, H_3), 4.50 (t, J_{OH-5} = 5.5 Hz, 1, 5-*OH*), 4.89 (d, J_{OH-3} = 4.7 Hz, 1, 3-*OH*), 5.21 ("t", J_{1-2} ~ $J_{1-2'}$ ~ 4.3 Hz, 1, H_1), a small peak at δ 7.32 indicated the presence of less than 3% of presumed benzylated material in product *106*

Methyl 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-β-*D*-*erythro*-pentofuranoside (*107*).

p-Nitrobenzoylation of 0.200 g (1.35 mmoles) of *104* using 0.75 g (4.05 mmoles) of *p*-nitrobenzoyl chloride in 5 ml of dry pyridine was effected as described by Ness *et al.*197) Crystallization of the resulting syrup from 50 ml of boiling absolute EtOH gave 0.513 g (85%) of *107* as microcrystalline needles: mp 145-145.5°; $[\alpha]_D^{23}$ -6.2° (*c* 1.6, $CHCl_3$); nmr ($CDCl_3$) δ 2.41 (d of t, $J_{2'-1}$ = $J_{2'-3}$ = 5.1 Hz, $J_{2'-2}$ = 14 Hz, 1, $H_{2'}$), 2.61 (d of d of d, J_{2-1} = 2.5 Hz, J_{2-3} = 6.7 Hz, $J_{2-2'}$ = 14 Hz, 1, H_2), 3.38 (s, 3, OCH_3), 4.45-4.75 (multiplets, 3, H_4, H_5, $H_{5'}$), 5.7 (d of d, J_{1-2} = 2.5 Hz, $J_{1-2'}$ = 5.1 Hz, 1, H_1), 5.67 (m, J_{3-2} = 6.7 Hz, $J_{3-2'}$ = 5.1 Hz, J_{3-4} ~ 2.2 Hz, 1, H_3), 8.15-8.4 (m, 8, 3+5-$COC_6H_4NO_2$). Reported197) mp 143-144°C; $[\alpha]_D^{20}$ -5.4°

(*c* 1.16, $CHCl_3$).

Anal. Calcd for $C_{20}H_{18}N_2O_{10}$: *C*, 53.81, *H*, 4.06, *N*, 6.28. Found: *C*, 53.55; *H*, 4.01; *N*, 5.96.

iso-Propyl 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-β-D-*erythro*-pentofuranoside (108).

p-Nitrobenzoylation of 0.091 g (0.52 mmoles) of 106 using 0.295 g (1.59 mmoles) of *p*-nitrobenzoyl chloride in 2.5 ml of dry pyridine was effected as described by Ness *et al.*197) Crystallization of the resulting syrup from ether—pentane yielded 0.177 g (72.5%) of fine, pale yellow needles of 108, mp 105.5-106°C. A sample for analysis was recrystallized from ether—pentane: mp 105-105.5°C; $[\alpha]^{25}_{D}$ -16.5° (*c* 1.5, $CHCl_3$); nmr ($CDCl_3$) δ 1.17, 1.20 (d, d, *J* = 6.2 Hz, 6, $CH(CH_3)_2$), 2.3-2.7 (m, 2, H_2, H_2'), 3.96 (septet, *J* = 6.2 Hz, 1, $OCH(CH_3)_2$), 4.4-4.47 (multiplets, 3, H_4, H_5, H_5'), 5.53 (d of d, J_{1-2} = 3.5 Hz, $J_{1-2'}$ = 5.0 Hz, 1, H_1), 5.65 (m, $J_{3-2,2'}$ ~ 5.7 Hz, J_{3-4} ~ 2 Hz, 1, H_3), 8.1-8.45 (m, 8, 3+5-$COC_6H_4NO_2$). Reported:197) mp 103-104°C; $[\alpha]^{20}_{D}$ -16.4° (*c* 1.4, $CHCl_3$).

Anal. Calcd for $C_{22}H_{22}N_2O_{10}$: *C*, 55.69; *H*, 4.68; *N*, 5.91. Found: *C*, 55.40; *H*, 4.64; *N*, 5.75.

Reaction of 94 with 6-chloropurine.

To a solution of 3.22 g (6.25 mmoles) of 94 in

40 ml of dry CH_3CN was added 1.93 g (12.5 mmoles) of 6-chloropurine and 12.3 g (62.5 mmoles) of $BaCO_3$. The resulting mixture was protected from moisture by a Drierite drying tube and was stirred magnetically at 60°C. After 15 hr, tlc (pentane-MeOAc, 7:3) showed complete conversion of 94 (R_f ~ 0.45) to major spots at R_f's ~ 0.65 and 0.35 as well as a minor spot at R_f ~ 0.15. Both the R_f 0.35 and 0.15 spots showed strong absorptions when viewed under 254 nm uv light. The reaction mixture was allowed to cool to room temperature and was diluted with ether (200 ml) and then filtered through Celite. The filtrate was cooled in ice water and was extracted with ice cold solutions of saturated $NaHCO_3$ (2 x 50 ml), H_2O (2 x 50 ml) and saturated NaCl (2 x 50 ml). The organic phase was dried over Na_2SO_4, filtered and evaporated to yield a yellow syrup (3.06 g). This syrup was dissolved in a minimal volume of CH_2Cl_2 and applied to a short silica gel column (4.6 x 17 cm, 100 g) packed in CH_2Cl_2. The column was successively eluted with CH_2Cl_2 (300 ml), CH_2Cl_2—MeOH (99:1, 300 ml), CH_2Cl_2—MeOH (98:2, 300 ml) and CH_2Cl_2—MeOH (97:3, 500 ml) at a flow rate ~20 ml/min. The column eluate from 250-550 ml was collected and evaporated to give 0.67 g of crude, syrupy 2-S-benzyl-3,5-di-O-benzyl-2-thio-D-arabinal (103). The syrup was purified by prepara-

tive tlc on 2 plates (20 x 20 cm) which were developed in pentane—ether (7:3). The major band was eluted with $CHCl_3$—MeOH (92:8) and the eluate was evaporated. Coevaporation of the residue with ether (3 x 20 ml) gave 0.538 g (20.5%) of chromatographically homogeneous **103** as a colourless syrup: $[\alpha]_{\mathbf{D}}^{26}$ +102.1° (*c* 1.52, $CHCl_3$); nmr ($CDCl_3$) δ 5.20, 5.41 (ABq of doublets, $J_{5,5'-4}$ = 6.1 Hz, $J_{5-5'}$ = 10.0 Hz, 2, H_5, $H_{5'}$), 4.61, 4.77 (ABq, J_{GEM} = 12.7 Hz, 2, $2\text{-S}\underline{CH_2}C_6H_5$), 4.34 (d of d, J_{3-1} = 0.9 Hz, J_{3-4} = 3.0 Hz, 1, H_3), 4.48, 4.56 (s, s; 2, 2; $3\text{+}5\text{-O}\underline{CH_2}C_6H_5$), 4.62 (t of d, J_{3-4} = 3.0 Hz, $J_{4-5,5'}$ = 6.1 Hz, 1, H_4), 6.38 (d, J_{1-3} = 0.9 Hz, 1, H_1), 7.0-7.45 (m, 15, $3\text{+}5\text{-OCH}_2\underline{C_6H_5}$ and $2\text{-SCH}_2C_6H_5$); ms m/e 418.1624 (100, **M**, calcd for $C_{26}H_{26}O_3{}^{32}S$: 418.1603), 310 (2.6, $\mathbf{M}\text{-}C_7H_7OH$), 281 (10), 91 (~850, $C_7H_7{}^+$).

The column eluate from 1000-1400 ml was collected and evaporated to give 1.84 g of yellow syrup. Tlc (CH_2Cl_2:MeOAc, 99:1, developed 4 times), revealed the presence of major and minor uv absorbing spots at R_f's ~ 0.3 and 0.1 respectively, and also a weakly uv absorbing spot at R_f ~ 0.4 which was very narrowly separated from the R_f 0.3 spot. The components were separated by preparative tlc on 4 plates (20 x 40 cm) which were developed 4 times in CH_2Cl_2—MeOAc (99:1). The major uv absorbing band (R_f 0.3 spot on tlc) was eluted with $CHCl_3$—MeOH (9:1). The eluate was evapo-

rated and the residue was coevaporated with ether (3 x 20 ml) to yield 0.900 g (25%) of a mixture of the β and α-anomers of 6-chloro-9-(2-benzylthio-3,5-di-O-benzyl-2-deoxy-D-ribofuranosyl)purine (109) in a ratio ~9:1. Uv max (MeOH) 264 nm (ε 8,420), (HCl) 264 nm (ε 7,940); nmr ($CDCl_3$), peaks assigned to the β-anomer: δ 3.53 (s, 2, 2'-$SCH_2C_6H_5$), 3.49, 3.71 (ABq of doublets, $J_{5',5''-4'}$ ~ 3.6 Hz, $J_{5'-5''}$ ~ 10.5 Hz, 2, $H_{5'}$, $H_{5''}$), 3.92 (d of d, $J_{2'-3'}$ = 5.3 Hz, $J_{2'-1'}$ = 7.5 Hz, 1, $H_{2'}$), 4.16 (d of d, $J_{3'-2'}$ = 5.3 Hz, $J_{3'-4'}$ = 2.4 Hz, 1, $H_{3'}$), 4.33 ("q", $J_{4'-5',5''}$ ~ $J_{4'-3'}$ ~ 3 Hz, 1, $H_{4'}$), 4.42, 4.56 (ABq, J_{GEM} ~ 12 Hz, 2, 3' or 5'-$OCH_2C_6H_5$), 4.50, 4.64 (ABq, J_{GEM} = 11.7 Hz, 2, 3' or 5'-$OCH_2C_6H_5$), 6.28 (d, $J_{1'-2'}$ = 7.5 Hz, 1, $H_{1'}$), 6.8-7.5 (m, 15, 3'+5'-$OCH_2C_6H_5$ and 2'-$SCH_2C_6H_5$), 8.20, 8.64 (s, s; 1, 1; H_2, H_8); peaks assigned to the α-anomer: δ 6.58 (d, $J_{1'-2'}$ ~ 8 Hz, $H_{1'}$), 8.64, 8.69 (s, s; H_2, H_8); ms m/e 572.1669 (29, M, calcd for $C_{31}H_{29}N_4O_3{}^{32}S^{35}Cl$: 572.1650), 537 (4.9, M-Cl), 481 (13, M-C_7H_7), 450 (24, M-$C_8H_{10}O$), 418 (100, M-BH), 205 (55), 155 (95, BH_2), 91 (~970, $C_7H_7^+$).

Similar elution of the minor uv absorbing band (R_f 0.1 spot on tlc) gave 0.388 g (11%) of a mixture of the β and α-anomers of 6-chloro-7-(2-benzylthio-3,5-di-O-benzyl-2-deoxy-D-ribofuranosyl)purine (110) in a ratio ~4:1. uv max (MeOH) 268 nm (ε 5,980), (HCl)

267.5 nm (ε 6,200); nmr (CDCl$_3$), peaks assigned to the β-anomer: δ 3.40 (s, 2, 2'-S$\underline{CH_2}$C$_6$H$_5$), 3.3-3.75 (multiplets, 3, $\underline{H}_{2'}$, $\underline{H}_{5'}$, $\underline{H}_{5''}$), 4.04 (d of d, $\underline{J}_{3'-4'}$ = 1.5 Hz, $\underline{J}_{3'-2'}$ = 5.3 Hz, 1, $\underline{H}_{3'}$), 4.28 (br q, 1, $\underline{H}_{4'}$), 4.40, 4.57 (ABq, $\underline{J}_{GEM}$ = 12.0 Hz, 2, 3' or 5'-O$\underline{CH_2}$C$_6$H$_5$), 4.55, 4.69 (ABq, $\underline{J}_{GEM}$ = 12.0 Hz, 3' or 5'-O$\underline{CH_2}$C$_6$H$_5$), 6.64 (d, $\underline{J}_{1'-2'}$ = 8.5 Hz, 1, $\underline{H}_{1'}$), 6.75-7.5 (m, 15, 3' and 5'-OCH$_2\underline{C_6H_5}$ and 2'-SCH$_2\underline{C_6H_5}$), 8.52, 8.85 (s, s; 1, 1; $\underline{H}_2$, $\underline{H}_8$); peaks assigned to the α-anomer: δ 6.70 (d, $\underline{J}_{1'-2'}$ = 7.0 Hz, $\underline{H}_{1'}$), 8.85, 8.96 (s, s; $\underline{H}_2$, $\underline{H}_8$); ms m/e 572.1669 (12, $\underline{\underline{M}}$, calcd for $C_{31}H_{29}N_4O_3{}^{32}S^{35}Cl$: 572.1650), 537 (4.9, $\underline{\underline{M}}$-Cl), 481 (31, $\underline{\underline{M}}$-C$_7H_7$), 451 (27, $\underline{\underline{M}}$-C$_7H_7OCH_2$), 418 (51, $\underline{\underline{M}}$-BH), 245 (100, $\underline{\underline{BH}}$ + C$_7$H$_7$), 155 (39, $\underline{\underline{BH_2}}$), 154 (21, $\underline{\underline{BH}}$), 91 (~1200, $C_7H_7^+$).

Elution of the weakly uv absorbing band (R$_f$ 0.4 spot on tlc) gave 0.39 g (14%) of a colourless syrup which was tentatively identified as 2-benzylthio-3,5-di-$\underline{O}$-benzyl-2-deoxy-$\underline{\underline{D}}$-arabinose ($\underline{111}$): nmr (CDCl$_3$) δ 5.0-5.5 (broad multiplets, 1, α,β-$\underline{H}_1$), 7.31 (s, 15, 3+5-OCH$_2\underline{C_6H_5}$ and 2-SCH$_2\underline{C_6H_5}$). Collection and evaporation of the eluate from 750-1000 ml in the original column chromatography gave a further 0.46 g (~17%) of crude $\underline{111}$. A sample (65 mg, 0.15 mmoles) of pure $\underline{111}$ was dissolved in 2 ml of dry pyridine and treated with 0.2 ml (2.1 mmoles) of acetic anhydride and 10 mg (0.08 mmoles) of 4-dimethylaminopyridine to effect

acetylation of the 1-hydroxyl group. After stirring for 0.5 hr, 0.2 ml of H_2O was added and stirring was continued for a further 0.5 hr to hydrolyze the excess acetic anhydride. The reaction mixture was diluted with ether (15 ml) and washed with saturated $NaHCO_3$ solution, H_2O and saturated NaCl solution. The organic phase was evaporated and the residue was co-evaporated successively with EtOH, toluene and ether to give a yellow syrup (70 mg) of the 1-O-acetyl derivative (112): nmr ($CDCl_3$) δ 1.90, 2.00 (s, s; 3, α,β-$OCOCH_3$), 6.18, 6.27 (d, d; J_{1-2} ~ 1.2 Hz, 1.8 Hz, 1, α,β-H_1), 7.1-7.3 (m, 15, 3+5-$OCH_2C_6H_5$ and 2-SCH_2-C_6H_5).

9-(2-Benzylthio-3,5-di-O-benzyl-2-deoxy-α,β-D-ribofuranosyl)adenine (113).

Amination of 0.844 g (1.51 mmoles) of 109 was effected[226)] by treatment with ~10 ml of anhydrous ammonia in a stainless steel bomb. After stirring magnetically for 20 hr at room temperature the ammonia was allowed to evaporate. The residue was treated with CH_2Cl_2 (50 ml) and the resulting mixture was washed with H_2O (4 x 25 ml). Tlc ($CHCl_3$—MeOH, 95:5) of the organic phase showed complete conversion of 109 (R_f ~ 0.85) to a slower migrating spot (R_f ~ 0.3). The organic phase was evaporated and

the residue was coevaporated successively with EtOH (20 ml) and CH_2Cl_2 (3 x 20 ml) to give 0.80 g (98%) of crude 113 as a colourless, solid foam. A sample of the crude product (100 mg) was purified by preparative tlc ($CHCl_3$—MeOH 95:5, developed twice) to yield 92 mg of pure 113 as a colourless, solid foam: uv max (MeOH) 259.5 nm (ε 14,900), (HCl) 258.5 nm (ε 13,600), (NaOH) 259.5 nm (ε 14,700); nmr ($CDCl_3$), peaks assigned to the β-anomer: δ 3.54 (s, 2, 2'-$SCH_2C_6H_5$), 3.52, 3.72 (ABq of doublets, $J_{5',5''-4'}$ ~ 4.3 Hz, $J_{5'-5''}$ = 10.2 Hz, 2, $H_{5'}$, $H_{5''}$), 3.95-4.20 (multiplets, 2, $H_{2'}$, $H_{3'}$), 4.31 (br q, 1, $H_{4'}$), 4.40-4.65 (multiplets, 4, 3'+5'-$OCH_2C_6H_5$), 6.06 (br s, 2, 6-NH_2), 6.27 (d, $J_{1'-2'}$ = 6.4 Hz, 1, $H_{1'}$), 6.95-7.50 (multiplets, 15, 3'+5'-$OCH_2C_6H_5$ and 2'-$SCH_2C_6H_5$), 7.90, 8.30 (s, s; 1, 1; H_2, H_8), peaks assigned to the α-anomer: δ 6.62 (d, $J_{1'-2'}$ = 7.0 Hz, $H_{1'}$), 8.35, 8.39 (s, s; H_2, H_8), the ratio of β to α-anomers was ~9:1; ms m/e 553.2190 (6.0, M, calcd for $C_{31}H_{31}N_5O_3{}^{32}S$: 553.2148), 462 (27, M-$C_7H_7$), 431 (11, M-$C_7H_6S$), 418 (77, M-BH). 324 (27, 431-C_7H_7O), 310 (16, 418-C_7H_7OH), 164 (28, BH + CHO), 136 (100, BH_2), 135 (81, BH), 91 (~1020, $C_7H_7{}^+$).

7-(2-Benzylthio-3,5-di-O-benzyl-2-deoxy-α and β-D-ribofuranosyl)adenine (115 and 114).

Amination of 0.32 g (0.56 mmoles) of 110 was effected as described above in the conversion of 109 to 113. Tlc ($CHCl_3$—MeOH, 95:5) of the crude product (0.31 g) showed complete conversion of 110 (R_f ~ 0.6) to 114 R_f ~ 0.3) and 115 (R_f ~ 0.23). These two components were separated by preparative tlc ($CHCl_3$—MeOH, 96:4, developed twice) on 2 plates (20 x 20 cm). The major, faster migrating band was eluted with $CHCl_3$—MeOH (8:2). The eluate was evaporated and the residue was coevaporated with CH_2Cl_2 (3 x 20 ml) to yield 0.215 g (69.5%) of 114 as a colourless, solid foam. Crystallization of this material from acetone—ether gave (2 crops) 0.155 g (50.5%) of pale yellow prisms of 114: mp 94.5-96°C; uv max (MeOH) 271.5, 253 nm (sh) (ε 8,900, 7,100), (HCl) 274.5 nm (ε 12,000), (NaOH) 271.5 nm (ε 8,800); nmr ($CDCl_3$) δ 2.86, 3.25 (ABq, J_{GEM} = 13.7 Hz, 2, 2'-SCH_2 C_6H_5), 3.16 (d of d, $J_{2'-1'}$ ~ 9.2 Hz, $J_{2'-3'}$ ~ 6.2 Hz, 1, $H_{2'}$), 3.31, 3.85 (ABq of doublets, $J_{5',5''-4'}$ ~ 1.4 Hz, $J_{5'-5''}$ = 11.0 Hz, 2, $H_{5'}$, $H_{5''}$), 4.05-4.25 (m, 2, $H_{3'}$, $H_{4'}$), 4.27, 4.42 (ABq, J_{GEM} = 11.4 Hz, 2, 3' or 5'-$OCH_2C_6H_5$), 4.44, 4.79 (ABq, J_{GEM} = 11.8 Hz, 2, 3' or 5'-$OCH_2C_6H_5$), 5.46 (br s, 2, 6-NH_2), 5.72 (d, $J_{1'-2'}$ = 9.4 Hz, 1, $H_{1'}$), 6.7-7.5 (multiplets, 15, 3'+5'-$OCH_2C_6H_5$ and 2'-$SCH_2C_6H_5$), 8.07, 8.46 (s, s; 1, 1; H_2, H_8); ms m/e 553.2128 (1.0, M,

calcd for $C_{31}H_{31}N_5O_3{}^{32}S$: 553.2148), 462 (2.5, **M**-C_7H_7), 418 (97, **M**-BH), 281 (8.9), 135 (100, **BH**), 91 (~660, $C_7H_7^+$).

Elution of the minor, slower migrating band gave 47 mg (15%) of the α-anomer (*115*) as an amorphous solid. Crystallization of this material from acetone—ether gave 37 mg (12%) of *115* as a microcrystalline solid: mp 156-157°C; $[\alpha]_{\mathbf{D}}^{24}$ +7.1° (*c* 0.75, MeOH); uv max (MeOH) 272, 247 nm (sh) (ε 7,950, 6,900), (HCl) 267.5 nm (ε 11,350), (NaOH) 272, 247 nm (sh) (ε 8,100, 7,000); nmr ($CDCl_3$) δ 3.38 (m, 2, $\underline{H}_{5'}$, $\underline{H}_{5''}$), 3.62 (s, 2, $2'\text{-S}\underline{CH_2}C_6H_5$), 3.68 (t, $\underline{J}_{2'-3'}$ ~ $\underline{J}_{2'-1'}$ ~ 6.4 Hz, 1, $\underline{H}_{2'}$), 4.05-4.25 (m, 2, $\underline{H}_{3'}$, $\underline{H}_{4'}$), 4.44 (s, 2, 3' or $5'\text{-O}\underline{CH_2}C_6H_5$), 4.48, 4.62 (ABq, $\underline{J}_{GEM}$ = 11.8 Hz, 2, 3' or $5'\text{-O}\underline{CH_2}C_6H_5$), 5.31 (br s, 2, $6\text{-}\underline{NH_2}$), 5.75 (d, $\underline{J}_{1'-2'}$ = 6.3 Hz, 1, $\underline{H}_{1'}$), 7.06 (s, 5, $2'\text{-S}CH_2\underline{C_6H_5}$), 7.1-7.4 (m, 10, $3'+5'\text{-O}CH_2\underline{C_6H_5}$), 8.44, 8.73 (s, s; 1, 1; $\underline{H}_2$, $\underline{H}_8$); ms m/e 553.2145 (4.7, **M**, calcd for $C_{31}H_{31}N_5O_3{}^{32}S$: 553.2148), 4.62 (12, **M**-C_7H_7), 431 (7.6, **M**-C_7H_6S), 135 (100, **BH**), 91 (~730, $C_7H_7^+$).

Anal. Calcd for $C_{31}H_{31}N_5O_3S$: *C*, 67.24; *H*, 5.64; *N*, 12.65; *S*, 5.79. Found: *C*, 66.77; *H*, 5.59; *N*, 12.64; *S*, 5.77.

9-(3,5-Di-*O*-benzyl-2-deoxy-α,β-**D**-*erythro*-pentofuranosyl)adenine (*116*).

Desulphurization of 113 was effected according to the procedure reported by Goodman and coworkers.192,193) To a magnetically stirred solution of 0.545 g (0.99 mmoles) of 113 in 40 ml of dry DMF was added 11.2 g (wet weight, prewashed with dry DMF) of Raney Nickel. Hydrogen was bubbled gently through the mixture which was heated to 98-100°C for 1.5 hr with a 250 watt infra-red lamp. The hot reaction mixture was filtered through Celite and the filter pad was washed with hot DMF (2 x 25 ml) and boiling MeOH (4 x 25 ml). The combined filtrat was evaporated and the residue was coevaporated with toluene (100 ml). The residue was dissolved in acetone and the resulting suspension was filtered through Celite. The filtrate was evaporated to yield 0.254 g (60%) of crude, syrupy 116. This material was purified by preparative tlc on two plates (20 x 40 cm) developed twice in $CHCl_3$—MeOH (96:4). The major band was eluted with $CHCl_3$—MeOH (8:2), the eluate was evaporated and the residue was coevaporated with acetone (3 x 10 ml) to give 0.168 g (40%) of a colourless glass of 116: $[\alpha]_D^{24}$ -10.2° (c 1.06, MeOH); uv max (MeOH) 259 nm (ε 15,000), (HCl) 258.5 nm (ε 13,500), (NaOH) 259.5 nm (ε 14,500); nmr ($CDCl_3$), peaks assigned to the β-anomer: δ 2.55-2.75 (m, 2, $H_{2'}$, $H_{2''}$), 3.68 (m, 2, $H_{5'}$, $H_{5''}$), 4.25-4.45 (m, 2, $H_{3'}$, $H_{4'}$), 4.55 (s, 4, 3'+5'-$OCH_2C_6H_5$), 6.02 (br s, 2, 6-NH_2), 6.47 (t, $J_{1'-2'} = J_{1'-2''} = 6.9$ Hz,

1, $\underline{H}_1$'), 7.15-7.4 (m, 10, 3'+5'-$OCH_2C_6H_5$), 8.08, 8.31 (s, s; 1, 1; $\underline{H}_2$, $\underline{H}_8$), no peaks attributable to the α-anomer could be detected; ms m/e 431.1948 (2.6, **M**, calcd for $C_{24}H_{25}N_5O_3$: 431.1957), 340 (70, **M**-C_7H_7), 325 (22, **M**-C_7H_6O), 219 (45, 325-C_7H_6O), 164 (88, **BH** + CHO), 162 (26, **BH** + C_2H_3), 136 (100, **BH$_2$**), 135 (73, **BH**), 91 (~830, $C_7H_7^+$).

7-(3,5-Di-*O*-benzyl-2-deoxy-β-*D*-*erythro*-pentofuranosyl)adenine (*117*).

Desulphurization of a solution of 0.120 g (0.22 mmoles) of *114* in 20 ml of dry DMF by 1.8 g of Raney Nickel was effected as described above in the conversion of *113* to *116*. Tlc ($CHCl_3$—MeOH, 95:5) of the crude product showed approximately equal amounts of *117* (R_f ~ 0.45) and unreacted *114* (R_f ~ 0.55) as well as a minor spot at R_f ~ 0.07. These components were separated by preparative tlc on one plate (20 x 20 cm) developed twice in $CHCl_3$—MeOH (95:5). The slower migrating, major band was eluted with $CHCl_3$—MeOH (7:3) and the eluate was evaporated to yield 19 mg (20%) of *117* as a colourless glass: uv max (MeOH) 271 nm (broad, ε 8,000); nmr ($CDCl_3$) δ 2.47 (d of d, $\underline{J}_{2',2''-1'}$ = 7.1 Hz, $\underline{J}_{2',2''-3'}$ = 4.5 Hz, 2, $\underline{H}_{2'}$, $\underline{H}_{2''}$), 3.57 (d of d, $\underline{J}_{5''-4'}$ = 2.6 Hz, $\underline{J}_{5''-5'}$ = 10.5 Hz, 1, $\underline{H}_{5''}$), 3.78 (d of d, $\underline{J}_{5'-4'}$ = 2.8 Hz, $\underline{J}_{5'-5''}$ = 10.5 Hz, 1, $\underline{H}_{5'}$), 4.25 (m,

1, $\underline{H}_{4'}$), 4.42 (m, 1, $\underline{H}_{3'}$), 4.43 (s, 2, 3' or 5'-$OC\underline{H}_2C_6H_5$), 4.46, 4.60 (ABq, $\underline{J}_{GEM}$ = 11.5 Hz, 2, 3' or 5'-$OC\underline{H}_2C_6H_5$), 5.85 (br s, 2, 6-$N\underline{H}_2$), 6.08 (t, $\underline{J}_{1'-2',2''}$ = 7.1 Hz, 1, $\underline{H}_{1'}$), 7.1-7.5 (m, 10, 3'+5'-$OCH_2C_6\underline{H}_5$), 8.00, 8.42 (s, s; 1, 1; $\underline{H}_2$, $\underline{H}_8$); ms m/e 431.1968 (2.2, M, calcd for $C_{24}H_{25}N_5O_3$: 431.1957), 340 (13, M-C_7H_7), 205 (37, 340-BH), 164 (24, BH + CHO), 162 (5.1, BH + C_2H_3), 136 (34, BH_2), 135 (100, BH), 91 (~560, $C_7H_7^+$).

Elution of the faster migrating, major band and evaporation of the eluate gave 21 mg (18%) of unreacted 114 as a colourless glass.

9-(2-Deoxy-α and β-D-erythro-pentofuranosyl)-adenine (α and β-2'-deoxyadenosine) (62 and 10).

A solution of 0.148 g (0.343 mmoles) of 116 in 20 ml of 95% EtOH containing 0.3 ml of 3 M NaOH was hydrogenated at 60 psi for 4.5 hr over 0.15 g of 10% Pd/C catalyst. The reaction mixture was filtered through Celite, the filter pad was washed with boiling MeOH (3 x 10 ml) and the combined filtrate was evaporated. The hydrogenation was then repeated for a further 12 hr and the reaction mixture was worked up as described above. The resulting residue was dissolved in 10 ml of MeOH and stirred for 0.5 hr with 0.5 g (dry weight) of IRC-50 (H^+) weakly acid cation exchange resin to effect neutralization. The mixture was filtered

and the filtrate was evaporated. The residue was dissolved in a minimal volume of H_2O and applied to a column (1.4 x 47 cm, 70 ml) of Dowex 1-X2(OH^-) (200-400 mesh) packed in H_2O. The column was eluted with H_2O at a flow rate of 1 ml/min and fractions were collected every 10 min. Fractions 53-66 were combined, evaporated and then coevaporated with EtOH. The residual solid was dissolved in ~0.5 ml of hot MeOH and ~2 ml of ether was added. The resulting solution was boiled and, after addition of further portions of Et_2O and repeated boiling, a small amount of solid material separated. The suspension was centrifuged and the resulting light brown solid was washed with ether to give 1.9 mg (2.2%) of the microcrystalline α-anomer, 62: mp 209-212°C; mmp with authentic 62, 211-216°C; mmp with authentic 2'-deoxyadenosine (10), 175-188°C; uv max (H_2O) 259.5 nm (ε 12,800), (HCl) 259 nm (ε 11,900), (NaOH) 259.5 nm (ε 12,900); ms m/e 251.1021 (3.9, M, calcd for $C_{10}H_{13}N_5O_3$: 251.1018), 221 (0.95, M-CH_2O), 164 (5.0, BH + CHO), 162 (36, BH + C_2H_3), 136 (27, BH_2), 135 (100, BH), 108 (20, BH - HCN). Reported[228] mp 212-213.5°C; uv max (H_2O) 259.5 nm (ε 15,500).

Fractions 69-135 were combined, evaporated and coevaporated with EtOH to yield 63 mg of colourless, solid 10. Recrystallization of this material from

MeOH—ether gave (2 crops) 48 mg (56%) of colourless prisms of *10*: mp 194.5-196°C; mmp with authentic *10*, 194-195.5°C; $[\alpha]_{D}^{25}$ -27.6° (*c* 0.86. H_2O); uv max (H_2O) 259.5 nm (ε 15,400), (HCl) 258 nm (ε 14,250), (NaOH) 259.5 nm (ε 15,350); nmr (DMSO-d_6) 2.27 (d of d of d, $J_{2''-1'}$ = 6.0 Hz, $J_{2''-3'}$ = 2.8 Hz, $J_{2''-2'}$ = 13 Hz, 1, $H_{2''}$), 2.75 (d of d of d, $J_{2'-1'}$ = 7.7 Hz, $J_{2'-3'}$ = 6.0 Hz, $J_{2'-2''}$ = 13 Hz, 1, $H_{2'}$), 3.5-3.7 (m, 2, $H_{5'}$, $H_{5''}$), 3.91 (m, 1, $H_{4'}$), 4.44 (m, 1, $H_{3'}$), 5.15-5.35 (m, 2, 3-*OH*, 5-*OH*), 6.36 (d of d, $J_{1'-2'}$ = 7.7 Hz, $J_{1'-2''}$ = 6.0 Hz, 1, $H_{1'}$), 7.28 (br s, 2, 6-NH_2), 8.14, 8.37 (s, s; 1, 1; H_2, H_8); ms m/e 251 (2.7, *M*), 221 (2.9, *M*-CH_2O), 164 (8.5, *BH* + CHO), 162 (33, *BH* + C_2H_3), 136 (30, BH_2), 135 (100, *BH*), 108 (23, *BH* - HCN). Reported190) mp 188-190°C; $[\alpha]_{D}^{21}$ -26° (*c* 1, H_2O).

Anal. Calcd for $C_{10}H_{13}N_5O_3$: *C*, 47.80; *H*, 5.21; *N*, 27.88. Found: *C*, 47.59; *H*, 5.13; *N*, 27.91.

7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)adenine (*118*).

A solution of 17 mg (0.039 mmoles) of *117* in 2 ml of 95% EtOH containing 1 drop of 3 *M* NaOH was hydrogenated at 60 psi over 20 mg of 10% Pd/C catalyst. After 23 hr the reaction mixture was filtered through Celite and the filter pad was washed with boiling MeOH (3 x 10 ml). The combined filtrate was evaporated to

yield a colourless syrup of crude 118. Tlc of this material ($CHCl_3$—MeOH, 9:1, developed twice) showed a major spot for 118 (R_f ~ 0.08), which migrated more slowly than 10 (R_f ~ 0.33), as well as a very faint spot at the origin. The syrup was dissolved in a minimal volume of H_2O and applied to a column (0.8 x 19 cm, 8 ml) of Dowex 1-X2(OH^-) (200-400 mesh) packed in H_2O. The column was eluted with H_2O at a flow rate of 36 ml/hr and fractions were collected every 10 min. Fractions 19-31 were combined, evaporated and coevaporated with EtOH to yield 8.1 mg (82%, dried 14 hr at 0.1 mm and 78°C over P_2O_5) of microcrystalline 118: mp 203-206°C; $[\alpha]_{\underline{D}}^{24}$ -74° ($\underline{c}$ 0.4, MeOH); uv (MeOH) max 271 nm (ε 7,150), min 232 nm (ε 3,150), (NaOH) max 270 (ε 7,500), min 232.5 (ε 3,650), (HCl) max 262.5 nm (ε 11,100), (HCl → NaOH) max 269 nm (the spectra in HCl and HCl made basic with NaOH are characteristic of adenine). The methanolic optical rotation and uv samples were combined and evaporated. The residue was dissolved in boiling MeOH, filtered through Celite and crystallized from MeOH—ether. Filtration gave 6.2 mg of fine, colourless needles of 118: mp 211-212.5° (some decomposition); nmr (DMSO-$\underline{d}_6$) δ 2.3-2.6 (m overlapped by solvent peaks, $\underline{H}_{2'}$, $\underline{H}_{2''}$), 3.57 (m, 2, $\underline{H}_{5'}$, $\underline{H}_{5''}$), 3.90 ("q", $\underline{J}_{4'-3'}$ ~ $\underline{J}_{4'-5',5''}$ ~ 3.7 Hz, 1, $\underline{H}_{4'}$), 4.41 (br m, 1, $\underline{H}_{3'}$), 5.13 (br t, 1, 5'-$\underline{OH}$, 5.40

(br d, 1, 3'-*OH*), 6.31 (t, $J_{1'-2',2''}$ = 6.4 Hz, 1, $H_{1'}$), 6.92 (br s, 2, 6-NH_2), 8.21, 8.50 (s, s; 1, 1; H_2, H_8), (DMSO-d_6—D_2O) peaks at δ 5.13, 5.40 and 6.92 disappear, multiplets at δ 3.57 and 5.41 appear sharper; ms m/e 251.1024 (1.3, **M**, calcd for $C_{10}H_{13}N_5O_3$: 251.1018), 221 (0.98, **M**-CH_2O), 164 (2.6, **BH** + CHO), 162 (11, **BH** + C_2H_3), 136 (16, **BH**$_2$), 135 (100, **BH**), 108 (29, **BH** - HCN).

REFERENCES

1. F. Miescher, Hoppe-Seyler's Med. Chem. Unters., 4, 441 (1871).
2. R. Altmann, Arch. Anat. u. Physiol. Physiol. Abt., 524 (1889).
3. P. A. Levene and L. E. Bass, "Nucleic Acids," Chemical Catalogue Co., New York, 1931.
4. R. S. Tipson, Advan. Carbohyd. Chem., 1, 193 (1945).
5. G. R. Barker, Advan. Carbohyd. Chem., 11, 285 (1956).
6. A. Bendich, in "The Nucleic Acids," E. Chargaff and J. N. Davidson, Eds., Vol. I, Academic Press, New York, 1955, Chapter 3.
7. P. A. Levene and W. A. Jacobs, Ber., 42, 2475 (1909).
8. R. H. Hall, "The Modified Nucleosides in Nucleic Acids," Columbia University Press, New York, 1971.
9. P. A. Levene, J. Biol. Chem., 63, 653 (1925).
10. P. A. Levene and R. S. Tipson, J. Biol. Chem., 104, 385 (1934).
11. J. M. Gulland, E. R. Holiday and T. F. Macrea, J. Chem. Soc., 1639 (1934).
12. J. M. Gulland and E. R. Holiday, J. Chem. Soc., 765 (1936).
13. J. M. Gulland and L. F. Story, J. Chem. Soc., 259, 692 (1938).
14. P. A. Levene and R. S. Tipson, J. Biol. Chem., 94,

809 (1932); 97, 491 (1932); 101, 529 (1933).

15. D. M. Brown and B. Lythgoe, J. Chem. Soc., 1990 (1940).

16. B. Lythgoe and A. R. Todd, J. Chem. Soc., 592 (1944).

17. J. Davoll, B. Lythgoe and A. R. Todd, J. Chem. Soc., 833 (1946).

18. V. M. Clark, A. R. Todd and J. Zussman, J. Chem. Soc., 2952 (1951).

19. J. Davoll, B. Lythgoe and A. R. Todd, J. Chem. Soc., 967, 1685 (1948).

20. J. Baddiley, in "The Nucleic Acids," E. Chargaff and J. N. Davidson, Eds., Vol. I, Academic Press, New York, 1955, Chapter 4.

21. J. A. Montgomery and J. H. Thomas, Advan. Charbohyd. Chem., 14, 283 (1959).

22. J. J. Fox and I. Wempen, Advan. Carbohyd. Chem., 17, 301 (1962).

23. A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press, New York, 1963.

24. C. A. Dekker and L. Goodman, in "The Carbohydrates," 2nd Ed., Vol. IIA, W. Pigman and D. Horton, Eds., Academic Press, New York, 1970, Chapter 29.

25. D. M. Brown and A. R. Todd, in "The Nucleic Acids," E. Chargaff and J. N. Davidson, Eds., Vol. I, Academic Press, New York, 1955, Chapter 12.

26. R. M. S. Smellie, Prog. Nucleic Acid Res., 1, 27 (1963).

27. V. L. Florentiev and V. I. Ivanov, Nature, 228, 519 (1970).

28. G. L. Brown, Prog. Nucleic Acid Res., 2, 259 (1963).

29. Kin-Ichiro Miura, Prog. Nucleic Acid Res., 6, 39 (1967).

30. J. D. Watson and F. H. C. Crick, Nature, 171, 964 (1953).

31. L. Pauling and R. B. Corey, Arch. Biochem. Biophys., 65, 164 (1956).

32. M. W. Nirenberg and P. Leder, Proc. Nat. Acad. Sci. U.S.A., 52, 1521 (1964).

33. F. H. C. Crick, J. Mol. Biol., 19, 548 (1966).

34. F. H. C. Crick, Prog. Nucleic Acid Res., 1, 163 (1963).

35. H. G. Khorana, Fed. Proceedings, 24, 1473 (1965).

36. C. R. Woese, Prog. Nucleic Acid Res. Mol. Biol., 7, 107 (1967).

37. See articles in "The Mechanism of Protein Synthesis," Cold Spring Harbor Symposia on Quan. Biol., 34 (1969).

38. J. Lucas-Lenard and F. Lipmann, Ann. Rev. Biochem., 40, 409 (1971).

39. J. R. Warner, P. M. Knopf and A. Rich, Proc. Nat. Acad. Sci. U.S.A., 49, 122 (1963).

40. J. N. Davidson, "The Biochemistry of the Nucleic Acids," 6th Ed., Methuen and Co. Ltd., London, 1969.

41a. F. Skoog, F. M. Strong and C. O. Miller, Science, 148, 532 (1965).

41b. F. M. Strong, "Topics in Microbial Chemistry," J. Wiley and Sons, Inc., New York, 1958.

42. C. O. Miller, Ann. Rev. Plant Physiol., 12, 395 (1961).

43. C. O. Miller, F. Skoog, M. H. Von Saltza and F. M. Strong, J. Am. Chem. Soc., 77, 1932 (1955).

44. C. O. Miller, F. Skoog, F. S. Okumura, M. H. Von Saltza and F. M. Strong, J. Am. Chem. Soc., 77, 2262 (1955).

45. R. H. Hall and D. S. DeRopp, J. Am. Chem. Soc., 77, 6400 (1955).

46. C. G. Skinner and W. Shive, J. Am. Chem. Soc., 77, 6692 (1955).

47. M. W. Bullock, J. J. Hand and E. L. R. Stockstad, J. Am. Chem. Soc., 78, 3693 (1956).

48. M. M. Baizer, J. R. Clark, M. Dub and A. Loter, J. Org. Chem., 21, 1276 (1956).

49. F. Skoog, H. Q. Hamzi, A. Szweykowska, N. J. Leonard, K. L. Carraway, T. Fujii, J. P. Helgeson and R. N. Leoppky, Phytochem., 6, 1169 (1967).

50. A. S. Belikov, A. I. Bankowsky and M. V. Tsarev, Zhur. Obsch. Khim., 24, 919 (1954).

51. N. J. Leonard and J. A. Deyrup, J. Am. Chem. Soc., 82, 6202 (1960).

52. R. Denayer, A. Cavé and R. Goutarel, Compt. Rend., 253, 2994 (1961).

53. A. Cavé, J. A. Deyrup, R. Goutarel, N. J. Leonard and X. G. Monseur, Ann. Pharm. Françaises, 20, 285 (1962).

54. G. Beauchesne and R. Goutarel, Physiologia Planatarum, 16, 630 (1963).

55. J. H. Rogozinska, J. P. Helgeson and F. Skoog, Physiologia Planatarum, 17, 165 (1964).

56. D. Klämbt, G. Thies and F. Skoog, Proc. Nat. Acad. Sci. U.S.A., 56, 52 (1966).

57. J. P. Helgeson and N. J. Leonard, Proc. Nat. Acad. Sci. U.S.A., 56, 60 (1966).

58. N. J. Leonard and T. Fujii, Proc. Nat. Acad. Sci. U.S.A., 51, 73 (1964).

59. H. Q. Hamzi and F. Skoog, Proc. Nat. Acad. Sci. U.S.A., 51, 76 (1964).

60. N. J. Leonard, S. Achmatowicz, R. N. Loeppky, K. L. Carraway, W. A. H. Grimm, A. Szweykowska, H. Q. Hamzi and F. Skoog, Proc. Nat. Acad. Sci. U.S.A., 56, 709 (1966).

61. D. S. Letham, Life Sci., 2, 569 (1963).

62. C. O. Miller, Proc. Nat. Acad. Sci. U.S.A., 47, 170 (1961).

63. D. S. Letham, Life Sci., 2, 152 (1963).

64. D. S. Letham, J. S. Shannon and I. R. C. McDonald, Tetrahedron, 23, 479 (1967).

65. G. B. Shaw, B. M. Smallwood and D. V. Wilson,

J. Chem. Soc., C, 921 (1966).

66. H. G. Zachau, D. Dütting and H. Feldmann, Ang. Chem. Int. Ed., 5, 422 (1966).

67. K. Biemann, S. Tsunakawa, J. Sonnenbichler, H. Feldmann, D. Dütting and H. G. Zachau, Ang. Chem. Int. Ed., 5, 590 (1966).

68. R. H. Hall, M. J. Robins, L. Stasiuk and R. Thedford, J. Am. Chem. Soc., 88, 2614 (1966).

69. M. J. Robins, R. H. Hall and R. Thedford, Biochemistry, 6, 1837 (1967).

70. J. T. Madison and H-K. Kung, J. Biol. Chem., 242, 1324 (1967).

71. M. Staehelin, H. Rogg, B. C. Baguley, T. Ginsberg and W. Wehrli, Nature, Lond., 219, 1363 (1968).

72. D. J. Armstrong, P. K. Evans, W. J. Burrows, F. Skoog, J. F. Petit, J. L. Dahl, T. Steward, N. J. Leonard and S. M. Hecht, J. Biol. Chem., 245, 2922 (1970).

73. W. J. Burrows, D. J. Armstrong, M. Kaminek, F. Skoog, R. M. Bock, S. M. Hecht, L. G. Bammann, N. J. Leonard and J. Occolowitz, Biochemistry, 9, 1867 (1970).

74. D. F. Babcock and R. O. Morris, Biochemistry, 9, 3701 (1970).

75. R. H. Hall, L. Csonka, H. David and B. McLennan, Science, 156, 69 (1967).

76. W. J. Burrows, D. J. Armstrong, F. Skoog, S. M. Hecht, J. T. A. Boyle, N. J. Leonard and J. Occolowitz, Biochemistry, 8, 3071 (1969).

77. J. Bartz, D. Söll, W. J. Burrows and F. Skoog, Proc. Nat. Acad. Sci. U.S.A., 67, 1448 (1970).

78. S. M. Hecht, N. J. Leonard, J. Occolowitz, W. J. Burrows, D. J. Armstrong, F. Skoog and R. M. Bock, Biochem. Biophys. Res. Comm., 35, 205 (1969).

79. A. J. Playtis and N. J. Leonard, Biochem. Biophys. Res. Comm., 45, 1 (1971).

80. H. J. Vreman, F. Skoog, C. R. Frihart and N. J. Leonard, Plant Physiol., 49, 848 (1972).

81. W. J. Burrows, D. J. Armstrong, F. Skoog, S. M. Hecht, J. T. A. Boyle, N. J. Leonard and J. Occolowitz, Science, 161, 691 (1968).

82. F. Harada, H. J. Gross, F. Kimura, S. H. Chang, S. Nishimura and U. L. RajBhandary, Biochem. Biophys. Res. Comm., 33, 299 (1968).

83. S. Nishimura, Y. Yamada and H. Ishikura, Biochim. Biophys. Acta, 179, 517 (1969).

84. B. Thimmappaya and J. D. Cherayil, Biochem. Biophys. Res. Comm., 60, 665 (1974).

85. H. Feldman, D. Dütting and H. G. Zachau, Z. Physiol. Chem., 347, 236 (1966).

86. R. H. Hall, "The Modified Nucleosides in Nucleic Acids," Columbia University Press, New York, 1971,

p. 257, 317.

87. R. H. Hall, Ann. Rev. Plant Physiol., 24, 415 (1973).

88. F. Skoog and D. J. Armstrong, Ann. Rev. Plant. Physiol., 21, 359 (1970).

89. D. J. Armstrong, W. J. Burrows, F. Skoog, K. L. Roy and D. Söll, Proc. Nat. Acad. Sci. U.S.A., 63, 834 (1969).

90. F. Fittler and R. H. Hall, Biochem. Biophys. Res. Comm., 25, 441 (1966).

91. M. L. Gefter and R. L. Russell, J. Molec. Biol., 39, 145 (1969).

92. M. D. Litwack and A. Peterkovsky, Biochemistry, 10, 994 (1971).

93. N. J. Leonard and S. M. Hecht, Israel J. Chem., 6, 539 (1968).

94. R. Y. Schmitz, F. Skoog, S. M. Hecht and N. J. Leonard, Phytochem., 10, 275 (1971).

95. N. J. Leonard, S. M. Hecht, F. Skoog and R. Y. Schmitz, Proc. Nat. Acad. Sci. U.S.A., 63, 175 (1969).

96. M. H. Fleysher, A. Bloch, M. T. Hakala and C. A. Nichol, J. Med. Chem., 12, 1056 (1969).

97. M. H. Fleysher, J. Med. Chem., 15, 187 (1972).

98. S. M. Hecht, N. J. Leonard, R. Y. Schmitz and F. Skoog, Phytochem., 9, 1173 (1970).

99. A. Myles and J. J. Fox, J. Med. Chem., 11, 143 (1968).

100. K. Rothwell and S. T. C. Wright, Proc. Roy. Soc., B, 167, 202 (1967).

101. R. Y. Schmitz, F. Skoog, S. M. Hecht, R. M. Bock and N. J. Leonard, Phytochem., 11, 1603 (1972).

102. S. M. Hecht, R. M. Bock, R. Y. Shcmitz, F. Skoog, N. J. Leonard and J. L. Occolowitz, Biochemistry, 10, 4224 (1971).

103. F. Skoog, R. Y. Schmitz, R. M. Bock and S. M. Hecht, Phytochem., 12, 25 (1973).

104. S. M. Hecht, R. M. Bock, R. Y. Schmitz, F. Skoog and N. J. Leonard, Proc. Nat. Acad. Sci. U.S.A., 68, 2608 (1971).

105. J. T. Grace, Jr., M. T. Hakala, R. H. Hall and J. Blakeslee, Proc. Am. Ass. Canc. Res., 8, 23 (1967).

106. R. Jones, Jr., J. T. Grace, Jr., A. Mittelman and M. W. Woodruff, Proc. Am. Ass. Canc. Res., 9, 35 (1968).

107. D. Suk, C. L. Simpson and E. Mihich, Proc. Am. Ass. Canc. Res., 9, 69 (1968).

108. B. Hacker and T. L. Feldbush, Biochem. Pharm., 18, 847 (1969).

109. M. P. Rathbone and R. H. Hall, Cancer Res., 32, 1647 (1972).

110. M. H. Fleysher, M. T. Hakala, A. Bloch and R. H. Hall, J. Med. Chem., 11, 717 (1968).

111. A. Bloch and C. A. Nichol, Proc. Am. Ass. Canc. Res., 9, 6 (1968).

112. Y. Rustum and E. Mihich, Cancer Res., 32, 1315 (1972).

113. H. K. Slocum and M. T. Hakala, Cancer REs., 35, 423 (1975).

114. B. Hacker, Biochim. Biophys. Acta, 224, 635 (1970).

115. H. E. Skipper, J. A. Montgomery, J. R. Thomson and F. M. Schabel, Jr., Cancer Res., 19, 425 (1959); 19, Part 2, 287 (1959).

116. W. A. H. Grimm and N. J. Leonard, Biochemistry, 6, 3625 (1967).

117. J. H. Lister, in "Fused Pyrimidines. Part II. Purines," Ed. by D. J. Brown, Wiley-Interscience, New York, 1971, p. 313.

118. D. M. G. Martin and C. B. Reese, J. Chem. Soc., C, 1731 (1968).

119. B. Shimizu and M. Miyaki, Chem. Pharm. Bull., 18, 570 (1970).

120. H. Vorbrueggen, E. Berger and U. Niedballa, Ger. Patent, 1,955,387; Chem. Abstr., 75, 49510d (1971).

121. P. Brooks, A. Dipple and P. D. Lawley, J. Chem. Soc., C, 2026 (1968).

122. A. Sivadjian, P. Sadorge, M. Gawer, C. Terrine and J. Guern, Physiol. Vég., 7, 31 (1969).

123. M. J. Robins, R. Mengel and R. A. Jones, J. Am.

Chem. Soc., 95, 4075, (1973).

124. M. Honjo, Y. Kanai, Y. Furukawa, Y. Mizuno and Y. Sanno, Biochim. Biophys. Acta, 87, 696 (1964).

125. Y. Furukawa, K. Kobayashi, Y. Kanai and M. Honjo, Chem. Pharm. Bull., 13, 1273 (1965).

126. M. J. Robins and S. R. Naik, Biochim. Biophys. Acta, 246, 341 (1971).

127. M. J. Robins, S. R. Naik and A. S. K. Lee, J. Org. Chem., 39, 1891 (1974).

128. C. A. Dekker, J. Am. Chem. Soc., 87, 4027 (1965).

129. R. A. Long, A. F. Lewis, R. K. Robins and L. B. Townsend, J. Chem. Soc., C, 2443 (1971).

130. T. P. Johnston, L. B. Holum and J. A. Montgomery, J. Am. Chem. Soc., 80, 6265 (1958).

131. J. S. Shannon and D. S. Letham, N.Z.J. Sci., 9, 833 (1966).

132. G. G. Deleuze, J. D. McChesney and J. E. Fox, Biochem. Biophys. Res. Comm., 48, 1426 (1972).

133. S. M. Hecht, A. S. Gupta and N. J. Leonard, Anal. Biochem., 30, 249 (1969); 38, 230 (1970).

134. S. M. Hecht, A. S. Gupta and N. J. Leonard, Biochim. Biophys. Acta, 182, 444 (1969).

135. K. Biemann and J. A. McCloskey, J. Am. Chem. Soc., 84, 2005 (1962).

136. S. J. Shaw, D. M. Desiderio, K. Tsuboyama and J. A. McCloskey, J. Am. Chem. Soc., 92, 2510 (1970).

137. S. M. Hecht, Anal. Biochem., 44, 262 (1971).

138. L. B. Townsend and R. K. Robins, J. Hetero. Chem., 6, 459 (1969).

139. M. J. Robins, J. R. McCarthy, Jr., R. A. Jones and R. Mengel, Can. J. Chem., 51, 131 (1973).

140. F. E. Hruska, A. A. Grey and I. C. P. Smith, J. Am. Chem. Soc., 92, 4088 (1970).

141. T. A. Khwaja and R. K. Robins, J. Am. Chem. Soc., 88, 3640 (1966).

142. N. J. Leonard, S. M. Hecht, F. Skoog and R. Y. Schmitz, Proc. Nat. Acad. Sci. U.S.A., 59, 15 (1968).

143. D. Semenow, C.-H. Shih and W. G. Young, J. Am. Chem. Soc., 80, 5472 (1958).

144. R. A. Long, A. F. Lewis, R. K. Robins and L. B. Townsend, J. Chem. Soc., C, 2443 (1971).

145. E. Fischer and B. Helferich, Chem. Ber., 47, 210 (1914).

146. P. A. Levene and H. Sobotka, J. Biol. Chem., 65, 469 (1925).

147. G. E. Hilbert and T. B. Johnson, J. Am. Chem. Soc., 52, 4489 (1930).

148. G. E. Hilbert and E. F. Jansen, J. Am. Chem. Soc., 58, 61 (1936).

149. G. A. Howard, B. Lythgoe and A. R. Todd, J. Chem. Soc., 1052 (1947).

150. J. Davoll and B. A. Lowy, J. Am. Chem. Soc., 73, 1650 (1951).

151. J. J. Fox, N. Yung, J. Davoll and G. B. Brown, J. Am. Chem. Soc., 78, 2117 (1956).

152. See selected preparations in "Synthetic Procedures in Nucleic Acid Chemistry," W. W. Zorbach and R. S. Tipson, Eds., Vol. I, Wiley-Interscience, New York, 1968, Section III.

153. K. Watanabe, D. Hollenberg and J. J. Fox, J. Carb. Nucleos. Nucleot., 1, 1 (1974).

154. U. Niedballa and H. Vorbrüggen, J. Org. Chem., 39, 3654, 3660, 3664, 3668, 3672 (1974).

155. J. Pliml and M. Prystaš, Adv. Hetero. Chem., 8, 115 (1967).

156. W. W. Zorbach, Synthesis, 329 (1970).

157. M. Hoffer, R. Duschinsky, J. J. Fox and N. Yung, J. Am. Chem. Soc., 81, 4113 (1959).

158. R. K. Ness and H. G. Fletcher, Jr., J. Am. Chem. Soc., 82, 3434 (1960).

159. J. J. Fox, N. C. Yung, I. Wempen and M. Hoffer, J. Am. Chem. Soc., 4066 (1961).

160. C. Pederson and H. G. Fletcher, Jr., J. Am. Chem. Soc., 82, 5210 (1960).

161. W. W. Zorbach and G. J. Durr, Jr., J. Org. Chem., 27, 1474 (1962).

162. J. Pliml, M. Pystaš and F. Šorm, Coll. Czech.

Chem. Comm., 28, 2588 (1963).

163. M. Prystaš, J. Farkaš and F. Šorm, Coll. Czech. Chem. Comm., 30, 3123 (1965).

164. T. Y. Shen, W. V. Ruyle and R. L. Bugianesi, J. Hetero. Chem., 2, 495 (1965).

165. M. P. Mertes, J. Zielinski and C. Pillar, J. Med. Chem., 10, 320 (1967).

166. M. Prystaš, and F. Šorm, Coll. Czech. Chem. Comm., 31, 1053 (1966).

167. G. J. Durr, J. F. Keiser and P. A. Ierardi, J. Hetero. Chem., 4, 291 (1967).

168. E. E. Leutzinger, W. A. Bowles, R. K. Robins and L. B. Townsend, J. Am. Chem. Soc., 90, 127 (1968).

169. K. V. Bhat and W. W. Zorbach, Carb. Res., 6, 63 (1968).

170. W. Hutzenlaub, R. L. Tolman and R. K. Robins, J. Med. Chem., 15, 879 (1972).

171. W. Lee, A. Martinez, L. Goodman and D. Henry, J. Org. Chem., 37, 2923 (1972).

172. S. Nesnow, T. Miyazaki, T. Khawaja, R. B. Meyer, Jr., and C. Heidelberger, J. Med. Chem., 16, 524 (1973).

173. P. T. Berkowitz, T. J. Bardos and A. Bloch, J. Med. Chem., 16, 183 (1973).

174. W. J. Woodford, B. A. Swartz, C. J. Pillar, A. Kampf and M. P. Mertes, J. Med. Chem., 17, 1027 (1974).

175. B. R. Baker, J. P. Joseph, R. E. Schaub and J. A. Williams, J. Org. Chem., 19, 1768 (1954).

176. G. A. Howard, J. Chem. Soc., 1045 (1950).

177. R. S. Wright, G. M. Tener and H. G. Khorana, J. Am. Chem. Soc., 80, 2004 (1958).

178. M. P. Kotick, C. Szantay and T. J. Bardos, J. Org. Chem., 34, 3806 (1969).

179. D. M. Brown, D. B. Parihar, C. B. Reese and A. R. Todd, J. Chem. Soc., 3035 (1958).

180. D. M. Brown, D. B. Parihar and A. R. Todd, J. Chem. Soc., 4242 (1958).

181. J. F. Codington, I. L. Doerr and J. J. Fox, J. Org. Chem., 29, 558 (1964).

182. A. Holý, Coll. Czech. Chem. Comm., 37, 4072 (1972).

183. A. Holý, Tetrahedron Letters, 1147 (1973).

184. A. Holý, Coll. Czech. Chem. Comm., 38, 100, 428 (1973).

185. R. A. Sanchez and L. E. Orgel, J. Mol. Biol., 47, 531 (1970).

186. M. M. Ponpipom and S. Hanessian, Can. J. Chem., 50, 246 (1972).

187. M. Ikehara and H. Tada, Chem. Pharm. Bull., 15, 94 (1967).

188. M. J. Robins and R. A. Jones, J. Org. Chem., 39, 113 (1974).

189. A. F. Russell, S. Greenberg and J. G. Moffatt, J. Am. Chem. Soc., 95, 4025 (1973).

190. C. D. Anderson, L. Goodman and B. R. Baker, J. Am. Chem. Soc., 81, 3967 (1959).

191. C. D. Anderson, W. W. Lee, L. Goodman and B. R. Baker, J. Am. Chem. Soc., 83, 1900 (1961).

192. W. W. Lee, A. Benitez, C. D. Anderson, L. Goodman and B. R. Baker, J. Am. Chem. Soc., 83, 1906 (1961).

193. K. J. Ryan, E. M. Acton and L. Goodman, J. Org. Chem., 36, 2646 (1971).

194. W. G. Overend and M. Stacey, Advan. Carbohyd. Chem., 8, 45 (1953).

195. S. Hanessian, Advan. Carbohyd. Chem., 21, 143 (1966).

196. W. G. Overend, in "The Carbohydrates. Chemistry and Biochemistry," 2nd Ed., Vol. IA, W. Pigman and D. Horton, Eds., Academic Press, New York and London, 1972, Chapter 9.

197. R. K. Ness, D. L. MacDonald and H. G. Fletcher, Jr., J. Org. Chem., 26, 2895 (1961).

198. K. V. Bhat and W. W. Zorbach, Carb. Res., 6, 63 (1968).

199. C. C. Bhat, K. V. Bhat and W. W. Zorbach, Carb. Res., 10, 197 (1969).

200. W. W. Zorbach and W. Bühler, Ann., 670, 116 (1963).

201. W. W. Zorbach, C. C. Bhat and K. V. Bhat, in "Deoxy Sugars," R. F. Gould, Ed., American Chemical Soc., Washington, 1968.

202. W. W. Zorbach, S. L. DeBernado and K. V. Bhat, Carb.

Res., 11, 567 (1969).

203. J. Pliml and F. Šorm, Coll. Czech. Chem. Comm., 32, 3060 (1967).

204. W. W. Zorbach and T. A. Payne, J. Am. Chem. Soc., 82, 4979 (1960).

205. C. P. J. Glaudemans and H. G. Fletcher, Jr., J. Am. Chem. Soc., 87, 2456, 4636 (1965).

206. K. Takiura and S. Honda, Carb. Res., 21, 379 (1972).

207. M. Haga and R. K. Ness, J. Org. Chem., 30, 158 (1965).

208. R. J. Ferrier and J. R. Hurford, Carb. Res., 38, 125 (1974).

209. T. Reichstein and E. Weiss, Advan. Carbohyd. Chem., 17, 65 (1962).

210. G. Casini and L. Goodman, J. Am. Chem. Soc., 86, 1427 (1964).

211. E. J. Reist and S. L. Holton, Carb. Res., 9, 71 (1969).

212. T. van Es, Carb. Res., 21, 156 (1972).

213. P. Brigl, Hoppe-Seyler's Z. Physiol. Chem., 122, 245 (1922).

214. A. Holý and F. Šorm, Coll. Czech. Chem. Comm., 34, 3383 (1969).

215. E. J. Reist, P. A. Hart, L. Goodman and B. R. Baker, J. Am. Chem. Soc., 81, 5176 (1959).

216. E. L. Hirst, J. K. N. Jones and E. Williams, J. Chem.

Soc., 1062 (1947).

217. R. K. Ness and H. G. Fletcher, Jr., J. Am. Chem. Soc., 80, 2007 (1958).

218. C. T. Bishop and F. P. Cooper, Can. J. Chem., 41, 2743 (1963).

219. S. Zen, S. Tashima and S. Kotō, Bull. Chem. Soc. (Japan), 41, 3025 (1968).

220. J. D. Stevens and H. G. Fletcher, J. Org. Chem., 33, 1799 (1968).

221. C. S. Hudson, J. Am. Chem. Soc., 31, 66 (1909).

222. J. Kuszmann and L. Vargha, Berichte, 96, 2327 (1963).

223. R. K. Robins and H. H. Lin, J. Am. Chem. Soc., 79, 490 (1957).

224. J. A. Montgomery and C. Temple, Jr., J. Am. Chem. Soc., 83, 630 (1961).

225. R. N. Prasad and R. K. Robins, J. Am. Chem. Soc., 79, 6401 (1957).

226. L. F. Christensen, A. D. Broom, M. J. Robins and A. Bloch, J. Med. Chem., 15, 735 (1972).

227. L. F. Christensen and A. D. Broom, J. Org. Chem. 37, 3398 (1972).

228. M. J. Robins and R. K. Robins, J. Am. Chem. Soc., 87, 4934 (1965).

B30138